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RESEARCH ARTICLE

Effects of photophase illuminance on locomotor activity, urine production and urinary 6-sulfatoxymelatonin in nocturnal and diurnal South African rodents

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ABSTRACT

Effects of photophase illuminance (1, 10, 100 and 330 lx of white incandescent lighting) on daily rhythms of locomotor activity, urine production and 6-sulfatoxymelatonin (6-SMT; 10 versus 330 lx) were studied in nocturnal Namaqua rock mice (*Micaelamys namaquensis*) and diurnal four-striped field mice (*Rhabdomys pumilio*). *Micaelamys namaquensis* was consistently nocturnal (~90–94% nocturnal activity), whereas considerable individual variation marked activity profiles in *R. pumilio*, but with activity mostly pronounced around twilight (~55–66% diurnal activity). The amplitude of daily activity was distinctly affected by light intensity and this effect was greater in *M. namaquensis* than in *R. pumilio*. Only *M. namaquensis* displayed a distinctive daily rhythm of urine production, which correlated with its activity rhythm. Mean daily urine production appeared to be attenuated under dim photophase conditions, particularly in *R. pumilio*. The results suggest that the circadian regulation of locomotor activity and urine production possesses separate sensitivity thresholds to photophase illuminance. *Micaelamys namaquensis* expressed a significant daily 6-SMT rhythm that peaked during the late night, but the rhythm was attenuated by the brighter photophase cycle (330 lx). *Rhabdomys pumilio* appeared to express an ultradian 6-SMT rhythm under both lighting regimes with comparable mean daily 6-SMT values, but with different temporal patterns. It is widely known that a natural dark phase which is undisturbed by artificial light is essential for optimal circadian function. Here, we show that light intensity during the photophase also plays a key role in maintaining circadian rhythms in rodents, irrespective of their temporal activity rhythm.

KEY WORDS: Photoperiodic species, Circadian rhythms, Activity, Urine production, Melatonin

INTRODUCTION

The lives of mammals are governed by a plethora of physiological and behavioural rhythms that oscillate on a daily and seasonal basis. Most of these rhythms are either directly or indirectly generated by a master biological pacemaker, which resides in the hypothalamic suprachiasmatic nucleus (SCN; Cassone et al., 1988; Ralph et al., 1990). Within neurons of the SCN, transcriptional/translational feedback loops of clock genes with near-24 h cycle lengths, form the backbone of self-sustained circadian oscillations (Reppert and Weaver, 2002). In fact, most cells throughout the body possess such genetically based circadian clocks (Yoo et al., 2004). However, for the internal time-keeping system to have an adaptive significance, it must be attuned to the solar day–night cycle. This is achieved most notably through the photic entrainment of the SCN cells and is initiated through ocular light exposure (Lucas et al., 2001). Although photic cues for circadian entrainment are projected fundamentally from melanopsin-expressing intrinsically photosensitive ganglion cells (ipRGCs), known also as non-image forming photoreceptors (NIFPs), recent studies have revealed the involvement of visual rods and cones in shaping the signal (Dkhissi-Benyahya et al., 2007; Altimus et al., 2010; van Diepen et al., 2013; Weng et al., 2013). This raises the important question of whether variations in visual photoreceptor compositions amongst different species impose distinct effects on the entrainment of the SCN.

SCN clock signals are transformed into downstream circadian rhythms through various output pathways. For example, one of the most commonly studied circadian rhythms, namely that of locomotor activity, is regulated by the dorsomedial nucleus that receives SCN input via the ventral subparaventricular zone (Saper et al., 2005). In another major output pathway, projections to the paraventricular nucleus extend to the sympathetic preganglionic neurons of the upper spinal column, from where the synthesis of pineal melatonin is regulated (Reiter et al., 2011). The cyclic secretion of melatonin subsequently disseminates photoperiodic information to peripheral tissues and plays a central role in the regulation of circadian adjustments and also seasonal adjustments in photoperiodic species (Reiter, 1993; Dubocovich and Markowska, 2005). Melatonin production is mostly induced by darkness, irrespective of whether a species is diurnal or nocturnal, and its main metabolite, 6-sulfatoxymelatonin (6-SMT), can easily be measured from urine to indicate the concentration of pineal secreted melatonin (Bojkowski et al., 1987; Challet, 2007). Furthermore, evidence suggests that species possess characteristic sensitivity thresholds in their circadian rhythms, such as the melatonin rhythm, to different light intensities and wavelengths. It is believed that these thresholds probably reflect the habitat to which the species are adapted and that it may accordingly also reflect specific visual adaptations of the species (Kumar and Rani, 1999; Peichl, 2005; Zubidat et al., 2009, 2010a,b).

The Namaqua rock mouse (*Micaelamys namaquensis*) and the four-striped field mouse (*Rhabdomys pumilio*) are two terrestrial species that occupy contrasting temporal niches. Locomotor activity is robustly entrained by the light–dark cycle in both of these species, with *M. namaquensis* expressing a strongly nocturnal activity rhythm and *R. pumilio* expressing a strongly diurnal activity rhythm, but with marked activity at dusk and dawn (Schumann

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et al., 2005; Skinner and Chimimba, 2005; van der Merwe et al., 2014). The retinal photoreceptor arrangements of these two species have previously been described as overall complementary to their temporal lifestyles. In addition, ipRGCs are observed at nearly equal densities in the two species (I.v.d.M., N.C.B., Á. Lukáts, V. Bláhová and P. Němec, unpublished data). Photic cues administered by the light–dark cycle are expected to prompt different physiological and behavioural reactions in these two species as a result of their contrasting temporal niches. In the present study, we set out to evaluate and compare their responses across a range of different photophase illuminance levels. Locomotor activity, urine production and urinary 6-SMT concentration were selected as parameters of rhythmicity because it is well recognized that these processes are regulated by the light–dark cycle through the SCN (Negoro et al., 2012).

MATERIALS AND METHODS

Animal housing

Nine adult wild *M. namaquensis* (Smith 1834) (four males, five females; mean body mass, 34.9 g) and nine adult wild *R. pumilio* (Sparrman 1784) (six males, three females; mean body mass, 35.1 g) were used in this experiment. *Micaelamys namaquensis* were captured in the rocky outcrops of the Soutpansberg region at Goro Game Reserve, Limpopo Province, South Africa (22°58′S, 29°25′E). *Rhabdomys pumilio* were live-trapped at Birha farms near Birha in the Eastern Cape Province, South Africa (33°22′S, 27°19′E). All animals were kept individually in semi-transparent plastic cages (58×38×36 cm) in a controlled room with an ambient temperature of 25±1°C and approximately 60% relative humidity. Each animal was given a small open plastic shelter and tissue paper for nesting material and had *ad libitum* access to food and water. Water and food (parrot seed mix; Marlton's, Durban, South Africa) were topped up and fresh food (apple and carrot) was replaced at random times every second or third day to avoid activity entrainment to the feeding schedule. Trapping permits were acquired (001-CPM403-00014, CRO95/12CR, CRO 96/12CR) and approval of all experimentation was given by the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa (EC063-11).

Experimental protocol

One incandescent lamp (white light, 100 W, Osram, Germany) was positioned centrally above every two neighbouring cages, approximately 50 cm above the cage floor level. The lights were coupled to a dimmer circuit, which permitted the manual adjustment of the light intensity. Illuminance was measured at the central cage floor level with a hand-held digital light meter (Major Tech, Johannesburg, South Africa; basic accuracy: ±5%+10 digit) and the dimmer adjusted to obtain the desired level of illuminance. A timer automatically switched the lights on at 06:00 h (start of photophase) and off at 18:00 h (start of scotophase) each day. The animals were consecutively exposed for a period of 21 days to each of four illuminance light cycles (ILCs): 1 lx ILC, 10 lx ILC, 100 lx ILC and 330 lx ILC; during this period, the illuminance of the photophases differed between the ILCs, but there was complete darkness during the scotophase. Locomotor activity was recorded throughout the second and third week while urine samples were collected throughout the 21st day of each ILC. Urine collection was used for the extrapolation of production rates across the ILCs and urine was also analysed for 6-SMT levels, which were compared between the 10 lx ILC (dim photophase) and the 330 lx ILC (bright photophase).

Locomotor activity: recording and data analysis

Activity was recorded by infra-red motion captors (Quest PIR internal passive infrared detector; Elite Security Products, Electronic Lines, London, UK) that were fixed centrally above each cage and detected movement over the entire cage floor area. The collective number of locomotory movements (activity counts) for each minute was stored on a computer using VitalView software (VitalView™, Minimitter Co., Sunriver, OR, USA). The activity data were analysed and the daily activity rhythms visually presented as double-plotted actograms using the computer program ActiView (ActiView™, Minimitter Co.). Activity counts and percentages of activity were compared between the four ILCs as well as within each ILC (photophase versus scotophase) for all of the mice within a species. The sums of the activity counts per photophase and per scotophase of each day were calculated for each individual across all four light cycles. These values were then used to estimate the mean number of activity counts (of all individuals combined) for each entire ILC as well as for the photophase and scotophase of each ILC separately. The number of activity counts during either the photophase or scotophase of each day was further expressed as a percentage against the total number of activity counts for each day and was calculated for all animals individually. The mean of these values within each of the ILCs was then presented as percentage of activity.

Urine production rate: collection and analysis

During the last day of each ILC (day 21), urine samples were collected from all tested individuals at 3 h intervals for the 24 h duration. For this, the animals were carefully transferred from the regular cages to modified cages. The modified cages matched the regular cages (semi-transparent, 58×38×36 cm) but had a stainless steel wire mesh floor fixed approximately 2 cm above the cage bottom. An open slit right below the stainless steel mesh floor, across the breadth of the cage, allowed the insertion of a plastic plate, which covered the cage floor and could be removed whenever the screened urine had to be collected. Urine was transferred to Eppendorf tubes using disposable glass Pasteur pipettes, weighed immediately after collection using a Mettler digital scale (Mettler, Zurich, Switzerland) and stored at −30°C until further analysis. When calculating urine volume (sample mass divided by urine specific gravity), urine specific gravity was assumed to be 1 g ml^{-1} (Schoorlemmer et al., 2001; Tendron-Franzin et al., 2004). Urine volume was converted to hourly urine production rate (µl h^{-1}) and the collective means are presented per species for each ILC as well as for the scotophase and photophase of each ILC separately. In addition, the mean values for all of the animals within a species were calculated at each 3 h point to present the 24 h rhythms in urine production rate.

Measurement of urinary 6-SMT concentration

Urinary 6-SMT levels were measured in six individuals from each species using a commercial enzyme-linked immunosorbent assay kit (IBL, Hamburg, Germany; cat. no. RE54031). In brief, 50 µl of diluted urine (1:50), enzyme conjugate and melatonin sulfate rabbit-antiserum was pipetted into microtiter wells and incubated for 2 h at room temperature. After washing, 100 µl of tetramethylbenzidine (TMB) substrate solution was added, followed by incubation for 30 min at room temperature; 100 µl stop solution was added and absorbance was measured at 450 nm. Samples were analysed in duplicate and the intra- and inter-assay coefficients of variation were 5.2–12.2% and 4.0–6.0%, respectively. 6-SMT values were corrected against urinary creatinine, which is a breakdown product from tissue proteins, usually formed by muscle tissues in mammals (Schmidt-Nielsen, 1997), and is excreted at a relatively constant

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Table 1. Locomotor activity (activity counts) and urine production rate during photophase and scotophase in *Micaelamys namaquensis* and *Rhabdomys pumilio* under different illuminance light cycles (ILCs)

		1 lx ILC	10 lx ILC	100 lx ILC	330 lx ILC
Locomotor activity					
M. namaquensis	Photophase	51.07±6.03	47.53±5.92	33.47±5.58	45.65±5.91
	Scotophase	438.00±28.79	608.39±39.63	551.29±35.97	671.22±43.66
	GLMM ($F_{1,985}$; P)	185.550; **0.00**	202.871; **0.00**	211.124; **0.00**	207.478; **0.00**
R. pumilio	Photophase	108.95±8.37	99.00±7.54	109.88±8.36	110.72±8.88
	Scotophase	73.90±5.74	77.32±5.89	86.35±6.57	89.54±7.01
	GLMM ($F_{1,957}$; P)	21.275; **0.00**	9.673; **0.00**	9.217; **0.00**	6.259; **0.01**
Urine production rate (µl h^{-1})					
M. namaquensis	Photophase	37.68±14.07	45.45±16.33	77.48±28.85	65.15±24.03
	Scotophase	125.72±44.59	106.46±37.54	125.12±44.68	112.42±39.75
	GLMM ($F_{1,186}$; P)	6.67; **0.011**	5.889; **0.016**	3.079; 0.081	3.867; 0.051
R. pumilio	Photophase	37.95±9.94	58.80±14.29	68.91±17.05	52.55±13.86
	Scotophase	28.85±7.51	34.61±8.76	69.99±17.70	70.66±18.92
	GLMM ($F_{1,173}$; P)	1.18; 0.279	4.622; **0.033**	0.005; 0.944	1.23; 0.269

Data are means±s.e.m. GLMM, generalized linear mixed model. Significant P-values (P<0.05) are in bold.

rate, so it can be used to adjust concentrations of hormone in urine. In brief, creatinine levels were obtained by pipetting 7 µl of urine into 210 µl of freshly prepared Picric reagent [1 vol. of saturated picric acid solution and alkaline Triton solution (4.2 ml Triton +12.5 ml 1 mol l^{-1} NaOH in 66 ml distilled water) in 10 vol. of distilled water] and incubated for 2 h in the dark at RT. Absorbance was measured at 492 nm.

Statistical analysis

Data and statistical analyses were performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and IBM SPSS Statistics version 21.0 (SPSS Inc., Chicago, IL, USA). Data were not normally distributed and hence were analysed for statistical significance by generalized linear mixed models (GLMMs); the *post hoc* least significant difference test was used where significant differences were detected. P<0.05 was considered significant and all values are expressed as means±s.e.m. The GLMMs tested for mean effects of illuminance on the different variables and for the interaction effects of illuminance with the phase of the day (photophase/scotophase), as well as the interaction effects of illuminance with the time of day (h), on the different variables. A gamma distribution with an identity link function was selected for statistical analyses of locomotor activity and urinary 6-SMT, whereas a gamma distribution with a log-link function was selected for analyses of urine production rate.

RESULTS

Locomotor activity

Namaqua rock mouse

Micaelamys namaquensis displayed a robust daily locomotor activity rhythm in accordance with the light–dark cycle and all individuals were invariably nocturnal. The mean activity count was significantly higher by night than by day (Table 1) and the percentage of nocturnal activity was similar across all ILCs (1 lx ILC: 89.56%; 10 lx ILC: 92.75%; 100 lx ILC: 94.28%; 330 lx ILC: 93.63%). The level of photophase illuminance had a significant effect on daily locomotor activity ($F_{3,985}$=7.883, P<0.001). The mean daily activity count of the 1 lx ILC (489.85±30.34) differed significantly from that of the 10 lx ILC (655.92±40.76; $F_{3,985}$=7.883, P<0.05), the 100 lx ILC (584.75±37.16; $F_{3,985}$=7.883, P<0.05) and the 330 lx ILC (716.87±44.68; $F_{3,985}$=7.883, P<0.001; Fig. 1). Furthermore, a

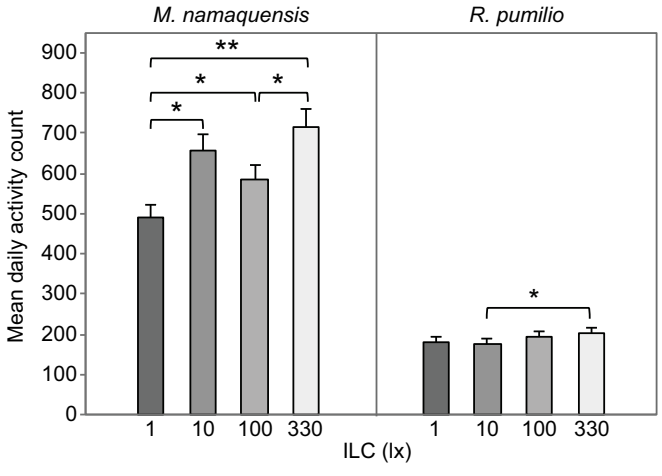

Fig. 1. Daily activity counts in *Micaelamys namaquensis* and *Rhabdomys pumilio* following exposure to four successive illuminance light cycles (ILCs). The photophase of each ILC had a different illuminance level (i.e. 1, 10, 100 and 330 lx), whereas all scotophases consisted of complete darkness (*P<0.05; **P<0.001). Means±s.e.m., N=9 *M. namaquensis* and N=9 *R. pumilio*.

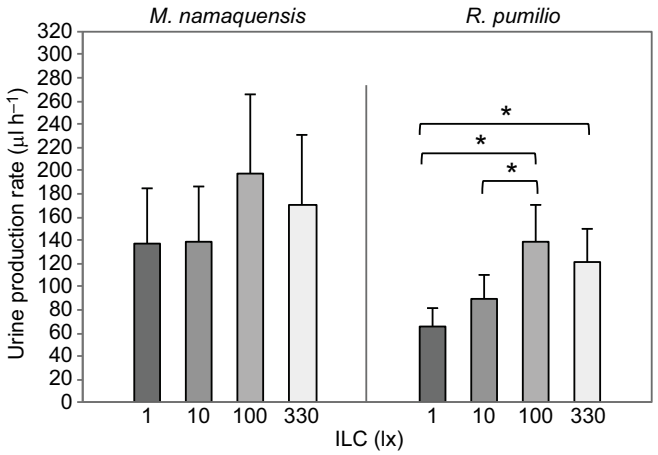

Fig. 2. Daily urine production rate in *M. namaquensis* and *R. pumilio* following exposure to four successive ILCs. The photophase of each ILC had a different illuminance level (i.e. 1, 10, 100 and 330 lx), whereas all scotophases consisted of complete darkness (*P<0.05; **P<0.001). Means±s.e.m., N=9 *M. namaquensis* and N=9 *R. pumilio*.

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significant difference was obtained between the mean daily activity count of the 100 lx ILC and the 330 lx ILC ($F_{3,985}$=7.883, P<0.05).

Four-striped field mouse

Rhabdomys pumilio was overall less active compared with *M. namaquensis* and its locomotor activity rhythm appeared to be less robust. A high degree of inter-individual variation was observed in the daily activity patterns (Fig. S1). Some mice were fundamentally crepuscular, some expressed activity intermittently throughout the 24 h day either with or without peaks around twilight, and yet others were mainly active during the photophase, but without any pronounced periods of activity around twilight. Consequently, the overall percentages of nocturnal activity (1 lx ILC: 40.41%; 10 lx ILC: 43.90%; 100 lx ILC: 44.00%; 330 lx ILC: 44.71%) were nearly equivalent to the percentages of diurnal activity. The mean activity count was significantly higher during photophase than scotophase under all of the ILCs (Table 1). The level of photophase illuminance had an overall significant effect on locomotor activity ($F_{3,941}$=2.759, P<0.05). The mean daily activity count was lowest during exposure to the 10 lx ILC (175.88±11.83) and this value differed significantly from the highest value, which was obtained under the 330 lx ILC (202.02±14.16; $F_{3,477}$=2.283, P<0.05). Under the 1 lx ILC and the 100 lx ILC, values were 180.52±12.24 and 196.22±13.2, respectively (Fig. 1).

Urine production rate

Namaqua rock mouse

Photophase illuminance had no significant influence on the mean daily urine production rate in *M. namaquensis* ($F_{3,186}$=1.335, P=0.264); values were similarly low under the 1 lx ILC (137.65±47.75 µl h^{-1}) and the 10 lx ILC (139.12±47.64 µl h^{-1}), highest under the 100 lx ILC (196.92±68.35 µl h^{-1}) and in-between under the brightest ILC (171.15±59.09 µl h^{-1}; Fig. 2). Urine production rates were consistently higher during the night-time than the daytime across all experimental groups, but only differed significantly between the 1 lx ILC and 10 lx ILC (Table 1). Daily rhythms in urine production rate for *M. namaquensis* are displayed in Fig. 3A. The overall interactive effect of photophase illuminance with the time of day did not produce a significant effect in any of the ILCs (1 lx ILC: $F_{7,186}$=1.073, P=0.382; 10 lx ILC: $F_{7,186}$=1.013, P=0.423; 100 lx ILC: $F_{7,186}$=0.839, P=0.556; 330 lx ILC: $F_{7,186}$=0.849, P=0.548). Under the 1 lx ILC, the peak value was obtained at 19:00 h (178.71±79.80 µl h^{-1}) and the lowest value at 13:00 h (20.22±9.03 µl h^{-1}). Under the 10 lx ILC, the highest and lowest values were at 04:00 h (116.79±48.95 µl h^{-1}) and 10:00 h (23.99±11.20 µl h^{-1}), respectively. Under the 100 lx ILC, the peak value was at 04:00 h (168.08±72.44 µl h^{-1}) and the lowest value

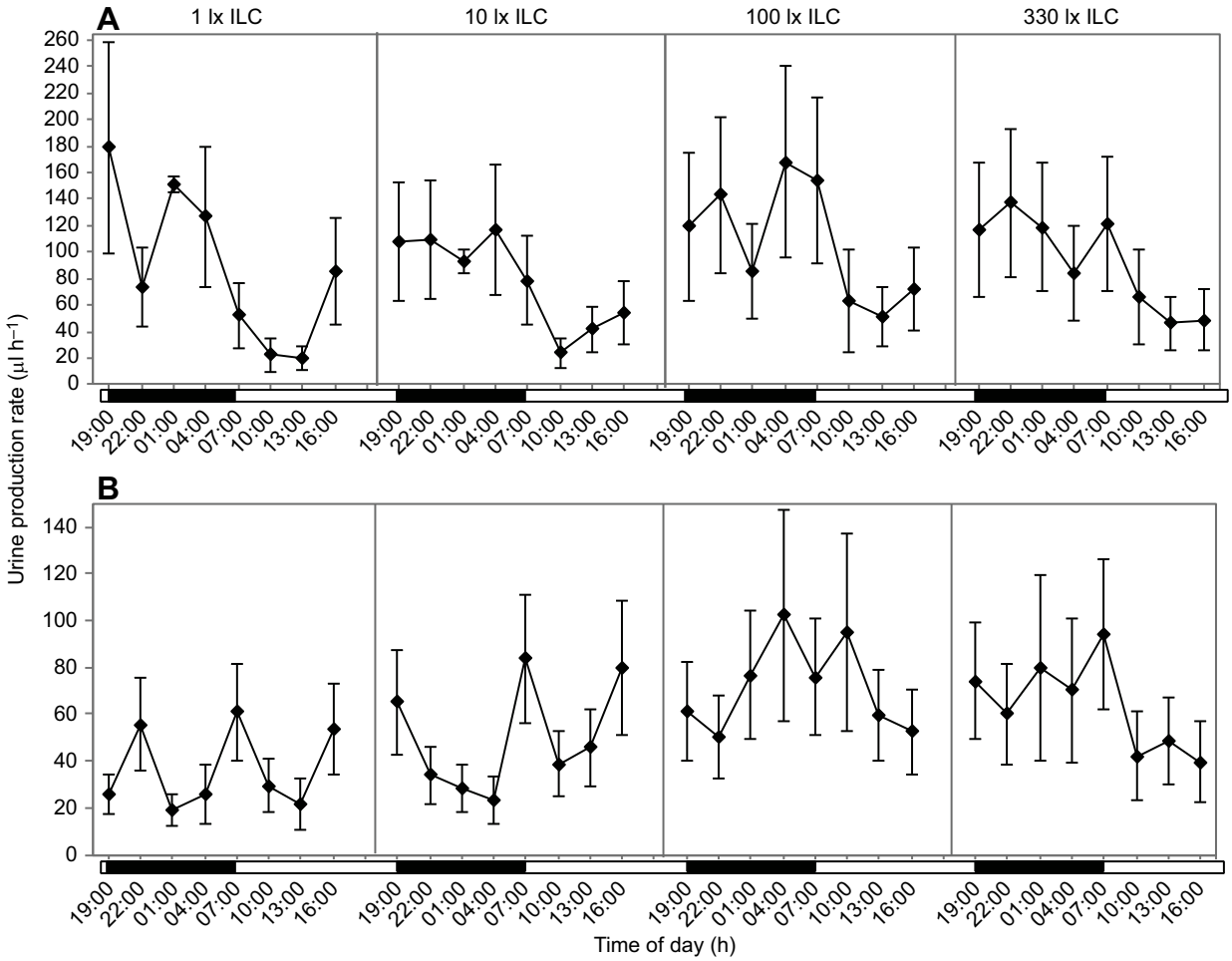

Fig. 3. The 24 h rhythms of urine production rate under four ILCs in *M. namaquensis* and *R. pumilio*. (A) *Micaelamys namaquensis* (*N*=9); (B) *R. pumilio* (*N*=9). The black and white bars at the bottom of each graph indicate the scotophase (18:00 h–06:00 h) and photophase (06:00 h–18:00 h) of each ILC.

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was at 13:00 h (51.69±22.23 µl h^{-1}). In the final experimental group, the rhythm peaked slightly earlier than during the preceding two ILCs (22:00 h: 137.13±56.18 µl h^{-1}) but the lowest value was again at 13:00 h (46.18±20.61 µl h^{-1}).

Four-striped field mouse

The effect of photophase illuminance on urine production rate was significant in *R. pumilio* ($F_{3,173}$=4.104, P=0.008). Exposure to the 1 lx ILC produced the lowest total mean urine production rate (66.18±15.31 µl h^{-1}), exposure to the 10 lx ILC produced a rate of 90.22±20.12 µl h^{-1}, the highest rate was obtained under the 100 lx ILC (138.90±31.15 µl h^{-1}) and under the 330 lx ILC the rate was 121.87±28.43 µl h^{-1} (Fig. 2). Mean urine production rates were significantly different between the 1 lx ILC and the 100 lx ILC ($F_{3,173}$=4.104, P=0.001), the 1 lx ILC and the 330 lx ILC ($F_{3,173}$=4.104, P=0.007) and the 10 lx ILC and the 100 lx ILC ($F_{3,173}$=4.104, P=0.019; Fig. 2). Urine production rate was higher during photophase than scotophase under the 1 lx ILC and significantly higher during photophase under the 10 lx ILC (Table 1). Under the 100 lx ILC, photophase and scotophase values were comparable and under the 330 lx ILC, the rate was slightly higher during scotophase (Table 1). The daily rhythms in urine production rate for *R. pumilio* are illustrated in Fig. 3B. The interactive effect of photophase illuminance with the time of day did not produce significant effects in any of the ILCs (1 lx ILC: $F_{7,173}$=1.189, P=0.311; 10 lx ILC: $F_{7,173}$=1.317, P=0.245; 100 lx ILC: $F_{7,173}$=0.464, P=0.860; 330 lx ILC: $F_{7,173}$=0.660, P=0.705). The daily urine production rhythms peaked consistently around dawn in all of the ILCs (Fig. 3B). During the 1 lx ILC and the 10 lx ILC, peak values were at 07:00 h (1 lx ILC: 60.81±20.70 µl h^{-1}; 10 lx ILC: 83.60±27.29 µl h^{-1}). Under the 100 lx ILC, the peak was slightly earlier (04:00 h: 102.22±45.34 µl h^{-1}), but during the 330 lx ILC it once again peaked at 07:00 h (93.97±32.00 µl h^{-1}).

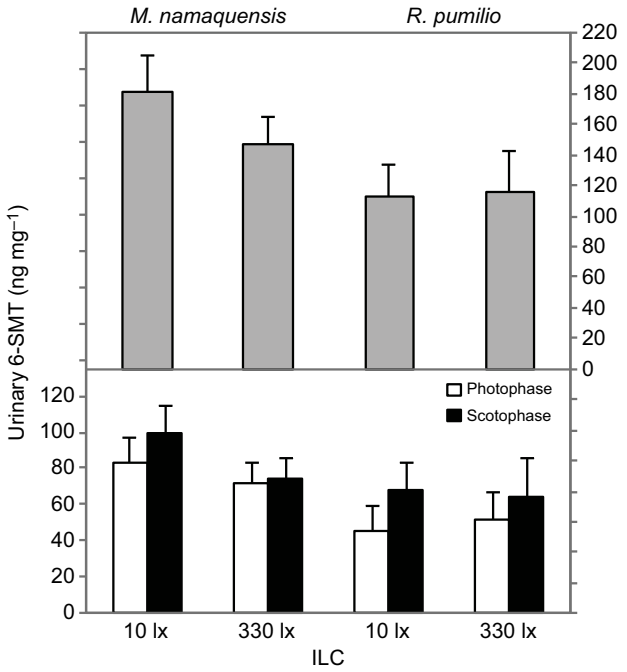

Fig. 4. Urinary 6-sulfatoxymelatonin (6-SMT) levels in *M. namaquensis* and *R. pumilio*. Levels of 6-SMT were measured under a dim ILC (10 lx ILC) and a bright ILC (330 lx ILC). Top: total levels; bottom, photophase and scotophase levels. Means±s.e.m., N=6 *M. namaquensis* and N=6 *R. pumilio*.

Urinary 6-SMT concentrations

Namaqua rock mouse

Urinary 6-SMT levels were higher under the 10 lx ILC (181.49±22.82 ng mg^{-1}) than for the 330 lx ILC (146.41±18.03 ng mg^{-1}; Fig. 4), though the effect was not significant ($F_{1,54}$=2.317, P=0.134). The interactive effect of photophase illuminance with the phase (light/dark) of the day did not show any significant effects under either of the ILCs but scotophase values were consistently higher than photophase values (Fig. 4). The 24 h 6-SMT profile for *M. namaquensis* is illustrated in Fig. 5. The interactive effect of photophase illuminance with the time of day significantly affected the urinary 6-SMT level during both ILCs (10 lx ILC: $F_{7,54}$=6.505, P<0.001; 330 lx ILC: $F_{7,54}$=6.039, P<0.001). During the 10 lx ILC, the lowest value of the rhythm was at 22:00 h (28.80±7.68 ng mg^{-1}), whereas the peak of the rhythm was at 04:00 h (262.41±55.28 ng mg^{-1}; Fig. 5). Exposure to the 330 lx ILC yielded a similar 24 h urinary 6-SMT rhythm, whereby again the lowest value was at 22:00 h (27.91±7.85 ng mg^{-1}) and the highest value was at 04:00 h (152.17±32.59 ng mg^{-1}; Fig. 5).

Four-striped field mouse

A non-significant effect ($F_{1,39}$=0.008, P=0.930; Fig. 4) was observed when comparing the response in urinary 6-SMT production under a low (10 lx ILC: 113.52±20.58 ng mg^{-1}) and a high (330 lx ILC: 116.42±25.54 ng mg^{-1}) photophase illuminance level. The interactive effect of photophase illuminance with the phase (light/dark) of the day was also not significant in both ILCs, but night-time 6-SMT values were consistently higher than daytime

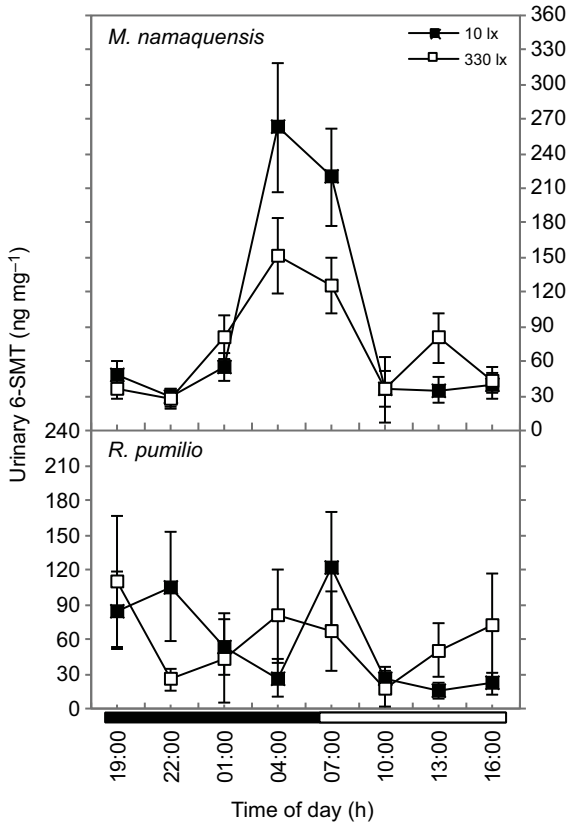

Fig. 5. The 24 h rhythms of 6-SMT in *M. namaquensis* and *R. pumilio* under a dim (10 lx) and a bright (330 lx) ILC. The black and white bars at the bottom of each graph indicate the scotophase (18:00 h–06:00 h) and photophase (06:00 h–18:00 h) of each ILC. Means±s.e.m., N=6 *M. namaquensis* and N=6 *R. pumilio*.

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values (Fig. 4). The 24 h 6-SMT profiles for *R. pumilio* are illustrated in Fig. 5. The interactive effect of photophase illuminance with the time of day had a non-significant influence on the level of urinary 6-SMT under the 10 lx ILC ($F_{7,39}$=1.887, P=0.098) and also a non-significant influence under the 330 lx ILC ($F_{7,39}$=1.030, P=0.426). Under the 10 lx ILC, there was a distinct increase in the urinary 6-SMT level at dusk (between (16:00 h and 19:00 h) that was followed by a further increase towards the first major peak (22:00 h: 105.43±46.91 ng mg^{-1}). A second, larger peak was observed at dawn (07:00 h: 121.23±48.25 ng mg^{-1}), whereas the lowest value was observed at 13:00 h (15.98±7.11 ng mg^{-1}). Exposure to the 330 lx ILC yielded a different 24 h 6-SMT profile in which the peak was around dusk (19:00 h: 110.51±56.78 ng mg^{-1}) and the lowest value was at 10:00 h (17.51±15.58 ng mg^{-1}).

DISCUSSION

Micaelamys namaquensis is fundamentally nocturnal while *R. pumilio* is essentially diurnal; consequently, the species' active phases coincide with different photoenvironments in their natural habitats. The present results suggest that the level of photophase illuminance plays a key role in regulating locomotor activity, urine production and melatonin expression in *M. namaquensis* and *R. pumilio*.

Daily rhythms in locomotor activity

Because the availability of environmental resources changes in concurrence with changing levels of illumination and spectral dominant wavelength across the day, the temporal layout of locomotor activity of a species is vital. Several studies suggest that species hold unique thresholds to different qualities of lighting and that these reflect the environments they are adapted to (Tast et al., 2001; Zubidat et al., 2009, 2010a,b). The advantage of distinguishing between light intensities within the boundaries of specific thresholds is best demonstrated in species that adjust their activity patterns according to the level of moonlight. For example, many nocturnal animals either avoid open areas or reduce their locomotor activity altogether in an attempt to decrease predation risks under ranges of lighting that are equivalent to increasing moonlight (Alkon and Saltz, 1988; Julien-Laferrière, 1997; Topping et al., 1999).

The present study indicates that *M. namaquensis* and *R. pumilio* possess distinct locomotor activity responses to varying photophase illuminance levels, which appear to be associated with their temporal niches. Under the range of lighting conditions used here, all individuals of *M. namaquensis* and most individuals of *R. pumilio* had well-defined daily locomotor activity rhythms. As expected, *M. namaquensis* consistently exhibited robust nocturnal activity, whereas a considerable amount of inter-individual variation marked the temporal expression of activity in *R. pumilio*. However, each individual retained its own characteristic locomotor activity rhythm across the range of ILCs. Under natural conditions, ambient illuminance levels vary considerably throughout the day and could reach approximately 110,000 lx during the brightest part of the day. Most small diurnal mammals refrain from being active during the middle of the day and often make use of pathways that run underneath vegetation cover, perhaps to escape not only the heat but also the bright levels of lighting. Although the photophase illuminance did not influence the timing of daily activity expression, it determined the overall amount thereof. This effect also appeared to be more pronounced in *M. namaquensis* than in *R. pumilio*. During its active phase and in its natural environment, *R. pumilio* faces greater fluctuations in the photoenvironment than *M. namaquensis*. Lighting conditions change radically not only at dawn and dusk, when *R. pumilio* exerts most of its activity, but also when, for example, the mice move between their darker underground burrows or nests and the outside field throughout the day. Therefore, *R. pumilio*'s expressed capacity to tolerate a wide range of illuminances is an extension of its more diurnal lifestyle and serves to preserve the stability and thus the adaptive capacity of its photically entrained circadian rhythm of locomotor activity. In contrast, under natural circumstances, *M. namaquensis* is exposed to a photoenvironment with smaller fluctuations while it is active by night; for example, the illuminance level during full moon nights only reaches approximately 2 lx (Vásquez, 1994). This probably explains the greater sensitivity of *M. namaquensis* to the range of photophase illuminances used in the present study. It also highlights that light exposure during the species' sleep phase could play an important role in regulating the extent to which animals are active at night. In humans, even low levels of light can be transmitted through closed eyelids. The spectral transmittance of light through the human eyelid is attenuated at short wavelengths, i.e. the spectral region to which the circadian system is most sensitive (Bierman et al., 2011), and it is reasonable to assume that the same is true in the studied species. Interestingly, a relatively small increase from 1 lx to 10 lx, also a relatively dim ILC, yielded an amount of daily locomotor activity comparable to that obtained under the 330 lx ILC. Therefore, *M. namaquensis* was able to distinguish day from night under 1 lx of photophase lighting, but at this level of illuminance, stimulation of the pathway resulting in the expression of activity was attenuated. A different correlation has previously been reported in another nocturnal species, the social vole (*Microtus socialis*), whereby the mean daily energy expenditure rate, which is an index of locomotor activity, decreased with increased photophase intensity (Bennett and Ruben, 1979; Zubidat et al., 2010a). In Macaque monkeys, distinct differences in activity onset times were observed in bright light compared with dim light (Takasu et al., 2002). However, the bright light presented was an order of magnitude higher than in the current study (3500 lx) and the mice in the current study may also require higher light intensities for clear-cut differences to be observed. However, in some cases, changes as small as 1 or 2 lx can induce changes in locomotor activity patterns (Kramer and Birney, 2001). Whether differences in the density and distribution of visual and non-visual retinal photoreceptors could be attributed to these species-related differences remains to be elucidated.

Urine production rate

Urine production fluctuates across the day not as a mere function of the daily eating and drinking patterns of an organism but as a function of the circadian timing system (Negoro et al., 2012). Several processes that are tied to urine production clearly exhibit daily or circadian variations and are essential to the well-being of organisms (Kamperis et al., 2004; Noh et al., 2011). For example, the circadian timing of a bladder gap junction protein (connexin43), which regulates changes in bladder capacity, is fundamental for preventing micturition during sleep and thus improves sleep quality (Negoro et al., 2012). Another example is that of the relationship between atypical circadian rhythms of arginine vasopressin, a hormone renowned for regulating renal water excretion in mammals, and nocturnal polyuria syndrome (Asplund, 1995; Rittig et al., 2008). The endogenous timing of urine production is thus expected to ensure that the highest rates coincide with the wake period rather than the sleep period. Evidence also suggests an effect of photophase intensity on the timing of urine production, which

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might be species specific. In social voles (*M. socialis*), for instance, significant daily rhythms of urine excretion were observed at higher as opposed to lower photophase light intensities, whereas in blind mole rats (*Spalax ehrenbergi*), the rhythms were significant under both dim and bright photophase intensities (Zubidat et al., 2009).

In the present study, the 24 h urine production rhythms in *M. namaquensis* largely correlated to the species' nocturnal activity rhythm. In contrast, *R. pumilo* lacked an obvious 24 h urine production rhythm, yet the highest rates consistently coincided with the start of the species' active phase. Interestingly, similar trends in the response of mean daily urine production rates across the varying ILCs were observed in the two species, but significant differences were only observed in *R. pumilio*. In both species, brighter photophase illuminances appear to have a stimulating effect on urine production, yet values peaked under the 100 lx ILC as opposed to the 330 lx ILC. This might suggest similar sensitivity thresholds in these two species. It is interesting to note that mean daily urine production and mean daily locomotor activity responses were uncorrelated. This could indicate that the circadian regulation of locomotor activity and of urine production each possess their own sensitivity threshold to the level of photophase illuminance. Knowing that daily urine production rhythms result from the integrative workings of several hormones (e.g. arginine vasopressin, aldosterone, plasma renin and natriuretic peptide), which are also regulated by the circadian timing system (Kamperis et al., 2004; Noh et al., 2011), it is possible that the regulatory pathways of each of these hormones respond differently to varying qualities of lighting and that this caused the dissociation between locomotor activity and urine production, quantitatively.

Urinary 6-SMT concentration

In rats, exposure to light of as low as 0.2 lx during the normal dark phase has been shown to reduce melatonin by 87%, compared with the 94% reduction by constant lighting (Dauchy et al., 1997). The present results indicate that the photophase illuminance level is an important component in the regulation of melatonin expression in both of the studied species. Only *M. namaquensis* showed significant interactive effects between the photophase illuminance and the time of day under both ILCs (10 and 330 lx). In *M. namaquensis*, 6-SMT values peaked at around 2 h before the lights went on. It is likely that the period between pineal melatonin production and catabolism until 6-SMT detection in the urine caused a slight (1–2 h) delay (Nowak et al., 1987). The peak in melatonin might thus have occurred slightly earlier and this could imply that the contrast between daytime and night-time melatonin values is slightly larger in *M. namaquensis* than revealed here. Similar peaks in the melatonin content during the late night have also been observed in different strains of mice and in hamsters (Panke et al., 1978; Goto et al., 1989). In *M. namaquensis*, brighter photophase lighting actually had a slight attenuating effect on the melatonin rhythm. A similar effect has been reported in the strictly fossorial blind mole rat (*S. ehrenbergi*), whereby the melatonin rhythm was weakened by brighter photophase lighting conditions, presumably as a result of an increased stress response in the animals under bright lighting (Zubidat et al., 2009). Although *M. namaquensis* is not fossorial like *S. ehrenbergi*, it still sleeps and rests in protected spaces such as between rock crevices that are sheltered from intense daytime illumination. For this reason, the attenuated melatonin levels under bright lighting conditions may be stress related. Nevertheless, the significant interactive effect between the photophase illuminance and the time of the day under the 330 lx ILC suggests that the photoreception system of the mice was not yet saturated at this intensity. Also, in contrast to what was observed in *M. namaquensis*, Afrotropical stonechats (*Saxicola torquota axillaris*) show higher melatonin levels (with activity peaking in the early and late night) when exposed to bright light (Kumar et al., 2007), but the light presented to the birds was orders of magnitude higher than that for the mice.

In *R. pumilio*, the intensity of the photophase lighting did not affect the overall amount of melatonin produced daily. Interestingly, two relatively different daily rhythms in 6-SMT were observed, but both rhythms appeared to be ultradian. Ultradian patterns of melatonin secretion have previously been observed in humans and in mice, and it has been suggested that two separate oscillators are foundational to these patterns (Wehr et al., 1995; Nakahara et al., 2003). Evidence also suggests that daytime and night-time melatonin are regulated by different processes. At night, neurological signals from the superior cervical ganglia stimulate the rise in pineal melatonin production via sympathetic nerves (Moore, 1996; Reiter et al., 2011). In CBA mice, application of tetrodotoxin, a Na^+ channel blocker, effectively suppresses neurological stimulation of pineal melatonin production by night, but not by day (Nakahara et al., 2003). It has therefore been suggested that daytime melatonin regulation primarily results from endogenous activation inside the pinealocyte (Li et al., 2000). The present results strongly indicate that daytime and night-time melatonin production are also regulated separately in *R. pumilio*; this is best demonstrated by the prominent rise in daytime 6-SMT levels under the bright ILC but not the dim ILC. Our results also reveal that the daytime illuminance level plays an essential role in regulating melatonin production. Furthermore, the relatively high level of 6-SMT during the day while mice were subjected to the bright ILC was not unusual as a similar pattern has been observed in $B6D2F_1$ mice (Li et al., 2000). Tast et al. (2001) determined that photophase has a relatively low threshold to light intensity (40 lx) in diurnal domestic pigs, such that further increases in light intensity do not have an additional effect on melatonin levels.

Conclusions

Light plays a vital role in the regulation of the circadian system. However, when it intrudes into the natural dark phase, it may lead to the disruption of circadian organization and desynchronization in timing of the different biological rhythms, which is associated with various health risks and the disturbance of ecological systems (Bird et al., 2004; Navara and Nelson, 2007; Haim and Portnov, 2013). Although the effects of night-time light exposure on circadian organization are widespread, few studies have investigated the effects of varying intensities of daytime lighting on photoentrainment. The present results indicate that *M. namaquensis* and *R. pumilio* adjust their daily patterns in locomotor activity, urine production and melatonin excretion according to the light–dark cycle. *Micaelamys namaquensis* and *R. pumilio* respond differently to varying photophase light intensities, probably because they are adapted to different temporal niches in their natural habitats, where they are exposed to very different photoenvironments. Here we show that the level of photophase illuminance is an essential element in the process of photic entrainment of physiology and behaviour, irrespective of whether the species is active by day or by night. Apart from light intensity, the dominant wavelength within the photophase also changes across the day and this should be studied in the future.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conception and design – I.v.d.M., M.K.O., A.H., N.C.B. Data collection, analysis and/or interpretation – I.v.d.M., M.K.O., A.G. Drafting the article – I.v.d.M. Revision of the article – M.K.O., A.H., A.G., N.C.B.

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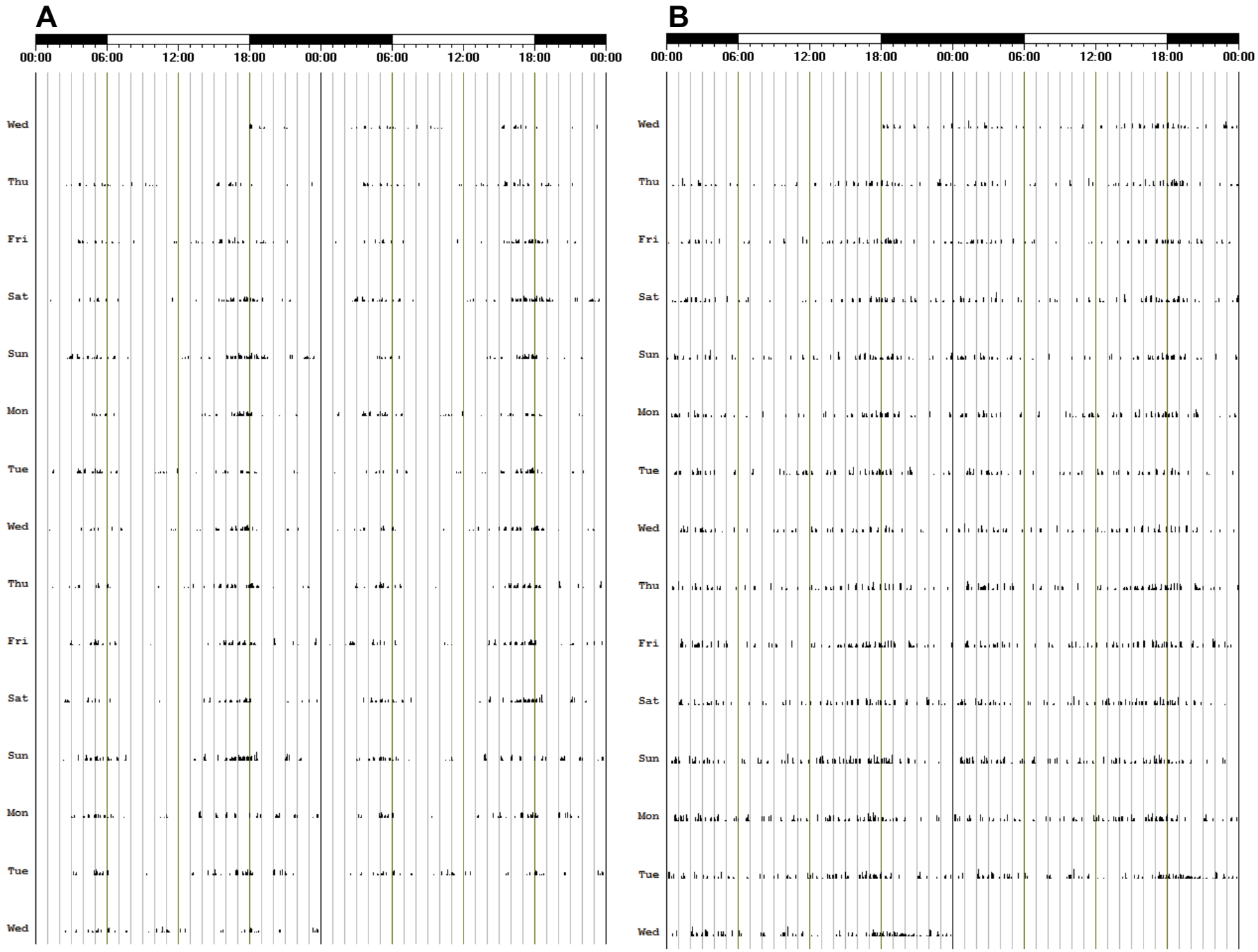

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Fig. S1. Representative actograms for *Rhabdomys pumilio*. The present actograms were recorded under the 1 lx ILC; in mouse number 2 (A), activity was concentrated around dusk and dawn whereas in mouse number 7 (B), activity was expressed intermittently throughout the day but with activity elevated around dusk and during the period between midnight and dawn.