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Increased Late Night Response to Light Controls the Circadian Pacemaker in a Nocturnal Primate

Martine Perret,1 Doris Gomez, Alexandra Barbosa, Fabienne Aujard, and Marc Théry
UMR 7179 Centre National de la Recherche Scientifique, Muséum National d’Histoire Naturelle, Département d’Ecologie et Gestion de la Biodiversité, Brunoy, France

Abstract  The mammalian endogenous circadian clock, the suprachiasmatic nuclei, receives environmental inputs, namely the light-dark cycle, through photopigments located in the eye and from melanopsin-expressing retinal ganglion cells. The authors investigated the influence of light wavelength and intensity on the synchronization of the rest-activity rhythm of the gray mouse lemur, a nocturnal Malagasy primate. Animals were tested at different irradiance levels (320, 45, 13, and 6 nmol.m−2.s−1) under several light wavelengths (from 400 to 610 nm). Several parameters including circadian period, activity, and body temperature waveforms were used to assess synchronization to a 12:12 light-dark cycle in comparison to control treatments (12:12 white light or continuous darkness). Entrainment of the circadian rest-activity cycle increased with light intensity. It was more efficient for mid wavelengths relative to shorter or longer wavelengths but not coincident with melanopsin maximal sensitivity, suggesting other photoreceptors are likely involved in lemurs’ photoentrainment. The authors obtained a novel synchronization pattern characterized by a clear synchronization to lights-on only without phasing to lights-off. Changes in photo-responsiveness at dusk and dawn highlight differential responses of evening and morning oscillators in the circadian clock.

Key words  circadian rhythm, light action spectrum, photo-responsiveness, natural entrainment, locomotor activity, nocturnal primate

Mammalian daily rest-activity rhythms are regulated by the major circadian clock located in the suprachiasmatic nuclei. The endogenous circadian rhythm of this clock, which usually deviates from a 24-h cycle, must be synchronized or regulated by environmental cues. The light-dark cycle has been identified as the most relevant of these cues. This photic entrainment has been experimentally shown to depend on photopigments located in the eye (Bowmaker, 2008; Doyle and Menaker, 2007).

A majority of eutherian mammals have two types of cones sensitive to short (SWS, near 430 nm) and long (M/LWS, beyond 500 nm) wavelengths, and one type of rod maximally sensitive to mid wavelengths, near 500 nm (Bowmaker, 2008). In addition, a subset of retinal ganglion cells (RGCs)

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1. To whom all correspondence should be addressed: Martine Perret, UMR 7179, Muséum National d’Histoire Naturelle, 1 avenue du petit château, 91800 Brunoy, France; e-mail: martine.perret@wanadoo.fr.

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contains melanopsin with a sensitivity peaking around 480 nm (Dacey et al., 2005; Lucas et al., 2001; Mawad and Van Gelder, 2008). Melanopsin-expressing RGCs project to numerous brain structures including the suprachiasmatic nuclei and are considered as the main mediator of circadian photoentrainment (Brainard et al., 2001; Doyle and Menaker, 2007; González-Menéndez et al., 2009; Rollag et al., 2003).

Circadian photoentrainment appears to depend on a complex interplay between photopigments: melanopsin-knockout mice still show photic entrainment (Mrosovsky, 2003; Ruby et al., 2002), light thresholds required for photic entrainment largely vary among nocturnal mammals (e.g., Erkert, 2008), and RGCs, rods, and cones all contribute to encode light intensity for photic entrainment (Dkhissi-Benyahya et al., 2007; Güler et al., 2008).

Monochromatic lights have been used to elicit circadian photoentrainment (Geetha and Subbaraj, 1996; Joshi and Chandrashekaran, 1985; Sharma et al., 1998; Takahashi et al., 1984). Blue or green light appeared more effective evoking phase shifts of locomotor activity, but with differential responses for phase advances or phase delays. In humans, different studies (synchronization of the circadian system, melatonin suppression, sleep regulation, and alertness) have revealed a higher efficiency of the 460- to 500-nm wavelength range, which corresponds more to melanopsin than to rod or cone sensitivity (Brainard et al., 2008; Cajochen, 2007; Lehrl et al., 2007; Lockley et al., 2006; Münch et al., 2006).

In nonhuman primates, despite some data suggesting the contribution of cones in circadian light perception (Deegan and Jacobs, 1996; Erkert et al., 2006), most studies have evaluated light thresholds required for triggering daily rest-activity rhythms (Erkert, 2008). This study explores for the first time the interactions between light intensity and wavelength on circadian photic entrainment of the rest-activity rhythm in a nonhuman primate, the gray mouse lemur *Microcebus murinus*. This nocturnal Malagasy primate stands as a convenient model species for such a study. First, this species has daily and seasonal activities that are strictly dependent on the photoperiod (Perret and Aujard, 2001b). Second, although from a basal clade, this strepsirrhine species presents many visual and behavioral characteristics that have been retained in further diverging primate lineages such as monkeys, apes, and humans (Blakeslee and Jacobs, 1985; Kay et al., 1997). Finally, there is detailed knowledge of its retina with low density of SWS and M/LWS cones and high density of rods (Dkhissi-Benyahya et al., 2001).

We tested different combinations of light intensity and wavelength and compared locomotor and temperature responses to control treatments. Results showed that the synchronization of the circadian rhythm increased with light intensity and was more effective under light of mid wavelengths. It also revealed differential patterns for dusk and dawn phasing according to light intensity and wavelength.

**MATERIALS AND METHODS**

**Animals**

Gray mouse lemurs *Microcebus murinus* were born and raised in the captive breeding colony created 40 years ago from a wild stock caught near the southern coast of Madagascar (CNRS/MNHN, Brunoy France, agreement B91.114.1). Animals are routinely kept at constant ambient temperature (24-26 °C) and hygrometry (55%-60%). They are fed ad libitum with a standardized diet including fresh fruit, a homemade milky mixture, and meal worms and kept under an artificial photocycle of 26 weeks of summer-like long-day photoperiod and 26 weeks of winter-like short-day photoperiod to ensure the seasonality of activity rhythms.

Thirty-four adult mouse lemurs (16 males and 18 females) from 2 to 4 years old were involved in this study. Before the experiments, animals were exposed for 1 month to an LD 12:12 winter-like short-days photoperiod, a condition that leads to pronounced fattening (104 ± 2.5 g for the animals tested), reduced activity, torpor, and complete sexual rest in both sexes (Perret and Aujard, 2001b). Winter reduction of behavioral activity was ideal to test synchronization to light, with minimal potential interference due to the activation of reproductive functions shown by males and females in long-day photoperiod (Perret and Aujard, 2001b). For 1 month before and during the experiment, animals were exposed to LD 12:12 and housed in individual cages (0.7 × 0.5 × 0.5 m) provided with a nest box and branches. They were placed in light-insulated ventilated chambers allowing constant ambient temperature (25 °C) and fine light control. All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC).

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Locomotor Activity and Body Temperature Parameters

Activity and temperature were estimated using telemetry. A small telemetric transmitter (2.5 g, Datascience, St Paul, MN) was implanted into the visceral cavity under ketamine anesthesia (IMALGENE, 100 mg/kg ip). After surgery, animals returned to their home cage and were allowed to recover for 1 month before recordings of locomotor activity and body temperature. Two receiver boards positioned at opposite sides of the cage continuously recorded locomotor activity and recorded temperature for 10 sec every 10 min (Dataquest Lab Pro, St Paul, MN). We then analyzed individual profiles over the last 7-day period of each test using ClockLab software (Actimetrics, Evanston, IL) and not over the last day only, which would have produced over- or underestimated parameters. We used the rapid increase or decrease in activity and body temperature to delineate nocturnal activity onset and offset in all tests performed (light exposure or free-running condition). Activity offset was delineated by a complete arrest of activity associated with a decrease of body temperature around 0.4 °C/10 min. The time of activity onset was defined as when counts of locomotor activity increased above the mean plus 2 SD of activity level recorded during the resting phase for at least 30 consecutive minutes, in association with a gradual increase in body temperature. The following parameters were analyzed: 1) the mean period tau (τ), length of time between consecutive activity onsets; 2) the mean \( \Psi_{\text{onset}} \) = absolute value of (time of lights-off—time of activity onset); 3) the mean \( \Psi_{\text{offset}} \) = absolute value of (time of lights-on—time of activity offset); 4) the duration of the resting phase (or subjective day)—length of time between activity offset and onset; 5) subjective night for free-running conditions—total amount of locomotor activity over the dark phase; 6) the activity performed during the first hour following activity onset; and 7) the last hour preceding activity offset as percentages of the total activity. To compare animals with different free-running periods, we normalized data by dividing them by the individual period lengths to give proportional measures. Finally, proceeding as in Aujard and Perret (2001a), we analyzed 8) the minimal body temperature (Tbmin) and 9) the time at which it was observed (Hmin), counting hours from time 12 taken as the beginning of the dark phase (or the subjective night).

Control Tests

Before the experiments, all animals were synchronized to a 24-h light-dark cycle (LD 12:12) using a strong white light (fluorescent daylight tube at 2600 µmol.m⁻².s⁻¹ as measured at the entrance of the nest, i.e., 820 cd.m⁻²) during the light phase and no light during the dark phase. Complete synchronization to the light/dark cycle was characterized by a mean period of 24 h, a long resting phase, phasing of activity patterns to lights-off and lights-on (both \( \Psi_{\text{onset}} \) and \( \Psi_{\text{offset}} \) lower than 15 min), a high activity level during the first and last hours of activity that accounted for 20% of the overall activity, and a torpor phase that started at light onset (Fig. 1, Table 1; Perret and Aujard, 2001a; 2001b).

At the end of the experiment, all animals were submitted to continuous darkness (LD 0:24) to record true free-running profiles. Nonsynchronized animals showed a circadian period shortened to 23 h, an increase of activity duration of about 130% with a decrease of the resting phase duration (on average 9 h 20 min), and low activity peaks at the beginning and end of the subjective night that accounted for 14% of the overall activity (Fig. 1, Table 1; Perret and Aujard, 2001a, 2001b).

Light Treatments

We spanned lemurs’ range of spectral sensitivity by using LED lights peaking at different wavelengths (spectrometric measurements in Fig. 2, Table S1 for LED characteristics): 401 nm (violet BestHongKong BUVC33W20WUVG), 431 nm (blue Kingbright L-53MBC), 474 nm (blue-green Nichia NSPB520S), 542 nm (green Nichia NSPG520S), 581 nm (yellow Boss type 34), and 610 nm (orange Kingbright L-53SED). We explored different light intensity values of 320, 45, 13, and 6 nmol.m⁻².s⁻¹ as measured at the entrance of the nest box placed 12.5 cm in front of the light source (see Table S1 for intensity values in lux or cd.m⁻²). We adjusted light intensity using a variable resistance and a spectrometer (Avantes AvaSpec-36.48, Eerbeek, the Netherlands) calibrated in intensity (using an Avalight DH-CAL light source) and connected through an optic fiber (FC-UV600-2-ME) to a CC3 cosine-corrected sensor. LED lights showed negligible shifts in wavelength peak with changes in light intensity. Moreover, all LEDs except the LED peaking at 431 nm had similar half peak bandwidth of approximately 30 nm and 60 nm, respectively (Supp. Table S1).
We chose light intensities tested after preliminary tests with white light conducted on 6 animals. Intensities of 320, 45, and 13 nmol.m^{-2}.s^{-1} (corresponding to 28.5, 4.0, and 1.1 lux, respectively) led to complete synchronization to the light-dark cycle. Only 2 of 6 animals were synchronized under the intensity of 6 nmol.m^{-2}.s^{-1} (0.57 lux, M. Perret, unpublished data, 2007). The lowest light intensity was chosen above the full moon light intensity measured with a sensor pointed toward the moon (direct measurement outside the Malagasy forest, 0.125 lux, computed from Pariente, 1980).

All tests consisted of exposing animals to LD 12:12, with a specific light treatment during the light phase and complete darkness during the nocturnal active phase. A focal test lasted at least 10 days to ensure a homogeneous response over a period of 7 days indispensable for data analysis. Animals were tested at decreasing intensities, for one (n = 21) or two (n = 13) different wavelengths. Between any two different wavelengths, animals were exposed to the control white light. We obtained data from 113 tests (see Table S1 for distribution of tests and animals). To avoid any possible entrainment of circadian rhythms by external cues, food was distributed in the dark, three times a week at random during the daily resting period.

Statistics

We analyzed data using generalized linear mixed models, suited to repeated observations on the same individuals and sample sizes varying across treatments (Bolker et al., 2009). We considered each change of light regime (lights-on and lights-off) as a separate synchronization event that we scored as a binary variable (0 or 1 for absence or presence of synchronization, respectively). A synchronization pattern was thus formed by two synchronization events. We found 3 different patterns (see Results section for description), namely, no synchronization (0,0), synchronization with advanced phasing to lights-off (0,1) and full synchronization (1,1). Considering events of synchronization and not patterns as the variable to explain, we analyzed synchronization as a function of change in light regimen (lights-on or lights-off), sex, light intensity, light dominant wavelength as fixed effects, and animal as random effect. We used a maximum likelihood approach and minimization of Akaike’s Information Criteria (AIC) to select the best statistical models according to the parsimony principle, considering that 2 models differing by less than 2 units of AIC are indistinguishable, as it is currently accepted (Burnham and Anderson, 1998). We corrected AIC values for potential residual

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**Table 1. Rhythm and locomotor activity parameters estimated for control treatments and exposure to different light spectra.**

<table>
<thead>
<tr>
<th></th>
<th>Control Responses</th>
<th>Responses to Light Spectra (LD 12:12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Synchronization</td>
<td>No Synchronization (Free-Run)</td>
</tr>
<tr>
<td>Period (min)</td>
<td>1440 ± 0.2</td>
<td>1378 ± 5.5^a</td>
</tr>
<tr>
<td>ψ_onset (min)</td>
<td>14 ± 1.7</td>
<td>396 ± 27^b</td>
</tr>
<tr>
<td>ψ_adjust (min)</td>
<td>6 ± 1.9</td>
<td>291 ± 28^a</td>
</tr>
<tr>
<td>Resting phase (min)</td>
<td>708 ± 1.9</td>
<td>590 ± 9.5^a</td>
</tr>
<tr>
<td>LA night (% total LA)</td>
<td>75.9 ± 2.1</td>
<td>82.2 ± 1.8^e</td>
</tr>
<tr>
<td>LA light-off (% LA night)</td>
<td>9.4 ± 0.6</td>
<td>7.0 ± 0.5^c</td>
</tr>
<tr>
<td>LA light-on (%LA night)</td>
<td>9.9 ± 1.0</td>
<td>6.9 ± 0.4^d</td>
</tr>
<tr>
<td>Tomin (min)</td>
<td>35.2 ± 0.2</td>
<td>35.5 ± 0.2</td>
</tr>
<tr>
<td>Hmin (min)</td>
<td>196 ± 9.3</td>
<td>212 ± 9.3</td>
</tr>
<tr>
<td>Number of tests</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Number of animals</td>
<td>34</td>
<td>34</td>
</tr>
</tbody>
</table>

Means ± SEM are mentioned for rhythm and locomotor activity parameters. Control responses under white light (synchronization) and continuous darkness (free-run). Responses to light spectra: synchronization, synchronization (S) with advanced phasing to lights-off (AS), and no synchronization (N). Parameters are indicated for comparisons (contrasts in linear mixed models associated with p < 0.05) to controls for white light (a) and free-run (b). LA = locomotor activity; Tomin = minimal body temperature; Hmin = hour at minimal temperature; ψ_onset = absolute delay between light-off and activity onset; ψ_adjust = absolute delay between light-on and activity offset.
overdispersion and small differences between number of parameters estimated and number of observations (Bolker et al., 2009). Coefficients and standard errors were computed using a restricted maximum likelihood approach and factor significance was tested using Wald z tests (Bolker et al., 2009).

Although a synchronization event was a binary response to a change in light regimen, we used the best model to compute the predicted synchronization expected under a light treatment. These fitted values ranging in the entire interval [0,1], they could be considered as synchronization probabilities, and interpreted as the proportion of synchronized animal in a population or as the probability that an animal would be synchronized under a light treatment. Finally, we explored the variations of activity and temperature parameters using a similar approach (see Supp. Table S2 for details about the models tested). All analyses were conducted using R (R Development Core Team, 2008).

RESULTS

Synchronization Patterns

When exposed to different light spectra, animals showed 3 different rest-activity rhythm patterns (Fig. 3, Table S1). First, 17/113 tests evoked complete synchronization to the light-dark cycle similar to that of white light controls (Figs. 1 and 3A): a period of approximately 24 h, phasing of activity to light cycle, and activity levels in the first and last hours of activity phase accounting for approximately 20% of the overall activity (Table 1). Although similar to white light controls in these crucial aspects, these fully synchronized patterns had significant deviations from
significant longer circadian period and smaller $\Psi_{\text{onset}}$ and $\Psi_{\text{offset}}$ values compared to free-run controls (Table 1).

Surprisingly, 39/113 tests induced a novel pattern that we defined as synchronization with advanced phasing to lights-off (referred to as AS, Fig. 3B). Animals presented a low $\Psi_{\text{offset}}$ value characteristic of a rapid stop of activity after lights-on, and a high $\Psi_{\text{onset}}$ value indicating an activity onset in complete dissociation with lights-off. The dissociation of activity onset from the lights-off took, on average, over $5.4 \pm 0.4$ days ($n = 15$) to reach a constant onset time. This pattern led to a significant decompression of activity phase, a reduction of the length of the resting phase, as well as a decrease in locomotor activity during the first hour of activity (Table 1). Yet, phasing to lights-on was coupled to a high level of locomotor activity during the last hour before activity offset and was sufficient to generate a period of approximately 24 h (Table 1). Phasing to lights-off was always associated to phasing to lights-on and led to a full synchronization to the light-dark cycle.

**Variation of Synchronization with Light Intensity and Wavelength**

The model that best explained the variations of synchronization included light intensity, light dominant wavelength, change in light regimen but not sex as significant factors. First, animals required less light intensity to stop than to start their activity, regardless of light dominant wavelength ($p < 0.0001$; Fig. 4). For instance, for mid wavelengths 474 or
Figure 4. Lemur synchronization to light according to light dominant wavelength (from 401 nm on the left to 610 nm on the right) and intensity (6 nmol.m⁻².s⁻¹: dark gray bars; 13 nmol.m⁻².s⁻¹: medium gray bars; 320 nmol.m⁻².s⁻¹: white bars) for activity offset (A) and onset (B). The synchronization probability (median as a dot, 1st and 3rd quartiles presented in box-and-whisker plots) was computed as the predicted values of the best model (see Statistics section for details). Notice that in plot B, the y-axis has been cut into 2 parts with different scales.

542 nm, the model predicted that the minimal light intensity required to achieve 50% synchronization (half of the animals expected to be synchronized) was 13 nmol.m⁻².s⁻¹ for lights-on but more than 45 nmol.m⁻².s⁻¹ for lights-off (Fig. 4). Second, for a given wavelength, synchronization linearly increased with light intensity, animals being better synchronized for higher intensities (p < 0.0001; Fig. 4). Finally, synchronization varied quadratically with light dominant wavelength: mid wavelengths (474 and 542 nm) were more effective to evoke phasing to light than intermediate wavelengths (431 and 581 nm), which in turn were more effective than extreme short or long (401 and 610 nm) wavelengths (p < 0.0001; Fig. 4). In other words, the light intensity required to elicit synchronization to a light regimen was lower for mid than for short or long wavelengths (Fig. 5). Wave-length efficiency to evoke synchronization was similar between the 2 wavelengths of a category (similar for 474 and 542 nm, for 431 and 581 nm, and for 401 and 610 nm; p > 0.05). Predicted values were slightly different from observed behaviors (proportions in Table S1) because the model corrected for the variations observed between light treatments that were due to the multiple use of the same animals.

Variation of Activity and Temperature Parameters with Synchronization Pattern, Light Intensity, and Wavelength

We confirmed that the categorization into 3 types of synchronization patterns was expressed in all parameters explored except locomotor activity preceding lights-on and time at minimal temperature (Table S2). Beyond this categorization, we observed that these parameters (especially the period and Ψ values) showed continuous variations between synchronization patterns and more interestingly within a synchronization pattern. For instance, nonsynchronized animals showed a period that increased with light intensity even if, overall, they constantly showed an absence of phasing to light cycle (Table S2). Parameters varied linearly with light intensity and quadratically with light wavelength. The wavelength 542 nm was slightly more efficient than 474 nm in eliciting a better phasing to light (lower Ψ values).
DISCUSSION

Our experiment revealed a novel synchronization pattern of the rest-activity rhythm. This particular synchronization is obtained by a differential response of activity onset and offset in relation to light wavelength and intensity. It is characterized by an activity offset clearly synchronized to lights-on and an absence of phasing to lights-off showing similarities with free-run patterns. The dissociation between lights-off and activity onset requires about 4 to 6 days to reach a constant onset. It occurs around 9 h 30 min after lights-on, which is similar to what we observed under free-running conditions. The duration of the resting period corresponds to the endogenous resting period of the animals. In nocturnal species, the restriction of locomotor activity to the dark phase of the light-dark cycle is explained by an inhibitory effect of light masking the circadian pattern (Erkert, 2008; Mrosovsky, 2003). In mouse lemurs, dim light exposure was insufficient to produce masking effects revealing the endogenous circadian pattern of activity onset. Nevertheless, the fact that several days were required to reach a stable pattern, while activity offset remained induced by lights-off of similar intensity, suggests differential photo-responsiveness at dawn and dusk.

The onset and offset of animal activity has been suggested to be controlled by 2 mutually coupled oscillators—E evening oscillator and M morning oscillator. This hypothesis formulated for nocturnal rodents (Pittendrigh and Daan, 1976) finds support in a large range of animals (e.g., Drosophila, Grima et al., 2004; humans, Wehr et al., 2001). Recent work has revealed that the suprachiasmatic nuclei (SCN) contain at least 2 separate oscillating cells with clock-related genes and proteins showing distinctive temporal expression patterns, particularly at dawn and dusk (Beaule et al., 2001; Daan et al., 2001; Hazlerigg et al., 2005; Herzog, 2007; Inagaki et al., 2007; Jagota et al., 2000). Research in Drosophila brain suggests that M and E oscillators are functionally coupled, although the M oscillator would be sufficient to drive the circadian rhythm (Grima et al., 2004; Picot et al., 2007; Stoleru et al., 2004). Our experiment shows that in mouse lemurs, the M oscillator seems sufficient to entrain the circadian rhythm. Whatever the wavelength, the M oscillator appears more sensitive than the E oscillator to light intensity. Interestingly, diurnal and nocturnal species studied to date share a more sensitive M than E oscillator (e.g., Grima et al., 2004; Pittendrigh and Daan, 1976) but differ in the behavioral transduction induced by the light signal (activity onset for diurnal, offset for nocturnal species).

Our study confirms that dark-light transitions are more powerful to entrain responses of the circadian system than light-dark transitions. Two nonexclusive processes may explain this result. First, photoreceptors may show a greater sensitivity to light during the dark phase (contrast perception, tonic-effect depending on prior light exposure, photoreceptor saturation) leading to different responses in the SCN (Boulos et al., 1996; Comas et al., 2008; Refinetti, 2007). Melanopsin-expressing cells in the retina show rhythmic oscillations with a greater expression just before lights-on (González-Menéndez et al., 2009). Second, transitions may be mediated by different photoreceptors, long-wavelength photoreceptors for dusk and short-wavelength photoreceptors for dawn entrainment (Joshi and Vanlalngahka, 2005). Although lemurs have photoreceptors sensitive to short and long wavelengths (Dkhissi-Benyahya et al., 2001), our results provide no direct support for the second hypothesis since the variation of synchronization with wavelength does not vary for dusk or dawn.

Our study reveals that light wavelength and intensity produce significant effects on both the waveform of the rest-activity rhythm and the duration of the circadian period. Locomotor activity parameters show continuous variations with light intensity (linear variation) and wavelength (quadratic variation). Although ineffective to entrain rest-activity rhythm, low light intensities alter the functioning of the circadian pacemaker, as observed in Syrian hamsters exposed to continuous dim green light (Evans et al., 2007). Time at minimal temperature shows no variation with light intensity, light wavelength, or synchronization pattern. The arousal from torpor and the return to normothermia are controlled by the circadian pacemaker and do not depend on light phototentrainment in the mouse lemur (Perret and Aujard, 2001a, 2001b).

Mid wavelengths are more efficient than short or long wavelengths to entrain the rest-activity rhythm at low light intensity (0.6-0.8 lux). This result echoes the numerous observations demonstrating that wavelengths around 460 to 500 nm are most powerful to entrain the circadian system (Cajochen, 2007; Doyle and Menaker, 2007; Geetha and Subbaraj, 1996; Lehrl et al., 2007; Lockley et al., 2006; Münch et al., 2006). Nevertheless, in our study, mouse lemurs seem maximally sensitive to a larger range of wavelengths 470 to 540 nm. The mid wavelengths tested,
474 and 542 nm, appear similarly efficient to control the duration of the circadian period and the synchronization to light cycle, with a slightly higher efficiency for 542 nm to induce phasing to light. This wavelength sensitivity does not coincide with melanopsin spectral absorption, suggesting that not only melanopsin but probably cones, particularly M/LWS cones, are likely involved in light photoentrainment (Fig. 5). The photopigments specifically involved in photoentrainment remain to be identified.

Our experiment shows that the most efficient wavelengths evoke synchronization for light intensities ranging from 0.5 to 1 lux (Table S1). This value is above the maximal light intensity at night (full moon at 0.1-0.2 lux outside the forest, computed from Pariente, 1980). It is comparable to dawn or dusk light intensity, which is at 0.1 to 1 lux in the Malagasy forest (Pariente, 1980). In nocturnal Malagasy prosimians including mouse lemurs, behavioral observations indicate that activity onset and offset correspond to early dawn and late dusk periods (e.g., Erkert, 2008). Twilight transitions are indeed used by most organisms as their primary environmental cue to adjust circadian phase (Boulos et al., 2002; Kay et al., 1997).

Data from other nocturnal mammalian species show a high variability of light intensity threshold values for behavioral photic entrainment or melatonin suppression: $10^{-5}$ lux in bats, 0.2 to 3 lux in rodents, and from 1 to 30 lux in nocturnal primates (Boulos et al., 2002; review in Erkert, 2008). The threshold value we obtained for the mouse lemur is presently the lowest value found in prosimian species. In mouse lemurs, the presence of a tapetum increases spectral sensitivity through higher photon capture efficiency. In addition to mechanisms for better scotopic acuity, high sensitivity for blue-green wavelengths enhanced by the tapetum would thus represent major components for the entrainment of circadian rhythms of mouse lemurs. Contrary to many nocturnal or marine mammals (review in Ahnelt and Kolb, 2000; Bowmaker, 2008) lemuriforms have conserved an S-opsin. The adaptive role of these opsins remains open and may be related to synchronization to light cycle, although our study does not suggest any predominant role for these cones.

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NOTE

Supplementary material for this article is available on the Journal of Biological Rhythms Web site at http://jbr.sagepub.com/supplemental.

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