

University of Groningen

## Nasal versus temporal illumination of the human retina

Ruger, M; Gordijn, Margaretha; Beersma, DGM; de Vries, Bontje; Daan, S

*Published in:*  
Journal of Biological Rhythms

*DOI:*  
[10.1177/0748730404270539](https://doi.org/10.1177/0748730404270539)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2005

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Ruger, M., Gordijn, M. C. M., Beersma, D. G. M., de Vries, B., & Daan, S. (2005). Nasal versus temporal illumination of the human retina: Effects on core body temperature, melatonin, and circadian phase. *Journal of Biological Rhythms*, 20(1), 60-70. DOI: 10.1177/0748730404270539

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

# Nasal versus Temporal Illumination of the Human Retina: Effects on Core Body Temperature, Melatonin, and Circadian Phase

Melanie Rüger,<sup>1</sup> Marijke C. M. Gordijn, Domien G. M. Beersma, Bonnie de Vries, and Serge Daan  
*Department of Behavioral Biology, University of Groningen, the Netherlands*

**Abstract** The mammalian retina contains both visual and circadian photoreceptors. In humans, nocturnal stimulation of the latter receptors leads to melatonin suppression, which might cause reduced nighttime sleepiness. Melatonin suppression is maximal when the nasal part of the retina is illuminated. Whether circadian phase shifting in humans is due to the same photoreceptors is not known. The authors explore whether phase shifts and melatonin suppression depend on the same retinal area. Twelve healthy subjects participated in a within-subjects design and received all of 3 light conditions—1) 10 lux of dim light on the whole retina, 2) 100 lux of ocular light on the nasal part of the retina, and 3) 100 lux of ocular light on the temporal part of the retina—on separate nights in random order. In all 3 conditions, pupils were dilated before and during light exposure. The protocol consisted of an adaptation night followed by a 23-h period of sustained wakefulness, during which a 4-h light pulse was presented at a time when maximal phase delays were expected. Nasal illumination resulted in an immediate suppression of melatonin but had no effect on subjective sleepiness or core body temperature (CBT). Nasal illumination delayed the subsequent melatonin rhythm by 78 min, which is significantly ( $p = 0.016$ ) more than the delay drift in the dim-light condition (38 min), but had no detectable phase-shifting effect on the CBT rhythm. Temporal illumination suppressed melatonin less than the nasal illumination and had no effect on subjective sleepiness and CBT. Temporal illumination delayed neither the melatonin rhythm nor the CBT rhythm. The data show that the suppression of melatonin does not necessarily result in a reduction of subjective sleepiness and an elevation of CBT. In addition, 100 lux of bright white light is strong enough to affect the photoreceptors responsible for the suppression of melatonin but not strong enough to have a significant effect on sleepiness and CBT. This may be due to the larger variability of the latter variables.

**Key words** human circadian photoreceptors, nasal illumination, temporal illumination, immediate effects, phase-shifting effects, melatonin, core body temperature, sleepiness

The mammalian eye contains a 3rd type of photoreceptor cell in addition to rods and cones (Berson, 2003). Animal studies proved these latter

receptors to be intrinsically photosensitive retinal ganglion cells (Berson et al., 2002), most of them containing the novel photopigment melanopsin (Hattar

---

1. To whom all correspondence should be addressed: Department of Behavioral Biology, University of Groningen, P.O. Box 14, Kerklaan 30, 9750 AA Haren, the Netherlands; e-mail: m.ruger@biol.rug.nl.

et al., 2002; Provencio et al., 2000). Response characteristics and output pathways show that these ganglion cells function as irradiance detectors. By transmitting the light signal directly from the retina to the SCN, the ganglion cells are involved in the entrainment of the circadian system to the light-dark cycle (Hattar et al., 2003; Panda et al., 2002; Ruby et al., 2002). They also contribute to the pupillary light reflex (Hattar et al., 2003; Lucas et al., 2001; Lucas et al., 2003) and play an important role in the suppression of melatonin by light (Lucas et al., 1999). Suppression of melatonin itself is associated with reduced nighttime sleepiness (Cajochen et al., 2000; Rüger et al., 2003) and elevation of body temperature (Badia et al., 1991; French et al., 1990; Rüger et al., 2003).

In humans, little is known about the nature, distribution, and density of the circadian photoreceptors in the retina. Brainard et al. (2001b) have shown that melatonin concentration is more suppressed by light of 505 nm than could be expected if the cone system (peaking at 555 nm) were responsible. In a further study, Brainard et al. (2001a) established an action spectrum that proved wavelengths of 446 to 477 nm (peak wavelength of 464 nm) to be most effective for melatonin suppression, as measured in plasma. This result was supported by Thapan et al. (2001), who also established an action spectrum for melatonin suppression. The authors concluded that their data support the involvement in the light-induced melatonin suppression of a nonrod, noncone photoreceptor. The action spectrum that fitted the data best followed a rhodopsin template with a wavelength of  $\lambda_{\max} = 459$  nm (Thapan et al., 2001). The findings on the involvement of the retinal ganglion cell system in melatonin suppression have recently been extended to circadian phase shifts. Lockley et al. (2003) demonstrated that blue light is more effective in inducing a phase shift of the biological clock than could be expected if the cone system were responsible for the phase shifts. Warman et al. (2003) showed that short wavelength (blue light) is as effective in inducing phase shifts as white light with a longer wavelength, although the white light contained more energy ( $4300 \mu\text{W}/\text{cm}^2$ ) than the short-wavelength light ( $28 \mu\text{W}/\text{cm}^2$ ). Gaddy et al. (1992, 1993) have shown that increasing corneal luminance is accompanied by increasing melatonin suppression in plasma and that pupil size does affect the amount of melatonin suppression significantly. Other studies focused on the distribution of circadian photoreceptors in the retina. Animal studies have revealed that circadian photoreceptors are not

homogeneously distributed over the retina (Cooper et al., 1993; Hannibal et al., 2002).

It would be of great interest to know whether this holds also for the human retina. Several functional studies have addressed this issue. Adler et al. (1992) did not find a significant difference in melatonin suppression between illumination of the central and the peripheral visual field with a stimulus of 1000 lux. Visser et al. (1999) showed that illumination of the nasal part of the human retina by a 4-h stimulus of 500 lux yielded a larger suppression of salivary melatonin than illumination of the temporal part, regardless of whether the upper or lower part of the retina was illuminated. Lasko et al. (1999) compared salivary melatonin suppression in response to 500 lux in either the upper or lower visual field for 3 h. The largest suppression of melatonin was obtained by the illumination of the upper visual field (the lower half of the retina). Glickman et al. (2003) explored the changes in plasma melatonin levels due to 90-min, 200-lux illumination of the superior retina, the inferior retina, and the full retina. In contrast to the studies of Visser et al. (1999) and Lasko et al. (1999), the pupils of the subjects were pharmacologically dilated during the light exposure to control for differences in pupil size. Again, the illumination of the inferior part of the retina caused the largest suppression of melatonin. This suggests either a higher density of receptors or a greater sensitivity per receptor cell in this part of the retina.

All these studies focused on the immediate effects of illumination of the retina on melatonin secretion. The studies varied in the stimulus used (wavelength, intensity, duration), the illuminated part of the retina (temporal, nasal, superior, inferior), and pupil size (dilated vs. undilated). We have now extended these investigations on the retinal distribution of the circadian photoreceptor system to circadian responses. We combined the immediate effects of light exposure on melatonin and core body temperature (CBT) with measurements of subjective sleepiness and tested whether light-induced phase shifts depend on the area of the retina that is exposed. We compared the immediate and phase-shifting effects of temporal and nasal retinal illumination to dim-light illumination (control condition) within 1 design. The timing of the light exposure was based on human phase response curves on ocular light pulses and was chosen in such a way that maximal delays were expected (Beersma and Daan, 1993; Honma and Honma, 1988; Khalsa et al., 2003; Minors et al., 1991).

## METHODS

### Subjects

A total of 7 female and 5 male healthy subjects (mean age = 21 years; SD  $\pm$  2.2 years) participated in the study. Subjects were screened by using a general health questionnaire and a Morningness-Eveningness Questionnaire (MEQ; Horne and Östberg, 1976). Only nonsmoking, nonextreme morning or evening types (i.e., subjects having MEQ scores between 31 and 69) were selected. Further exclusion criteria were eye problems such as farsightedness or shortsightedness, history of glaucoma or cataract, color blindness, and night blindness. Subjects could not have a history of psychiatric illness and could not have traveled more than 1 time zone in the previous month. Female subjects not using oral contraceptives were tested in the luteal phase of their menstrual cycle, whereas female subjects taking oral contraceptives were tested during the phase they took a contraceptive with a stable hormone concentration.

All subjects signed informed consent forms and were paid for their participation. The medical ethics committee of the University of Groningen approved the protocol.

### Time Isolation Facility

The protocol included 3 stays of 2.5 days each in the time isolation facility. The facility, where neither daylight nor clock information is present, can host 4 subjects simultaneously in separate rooms. Subjects could read or study, listen to music, watch videos, or perform other nonphysical activities. Light sources present in the isolation facility did not exceed 10 lux measured at eye level and direction of gaze at any position in the room.

### Experimental Protocol

The study took place from November 2002 to April 2003. The complete experimental protocol is summarized in Figure 1. Subjects entered the facility at 7 p.m. (day 0). Electrodes were fitted for recording an electroencephalogram (EEG; 2 channels, C3-A2 and Fz-A1), electro-oculogram, and electromyogram (EMG). At the same time, the test battery was introduced and explained, and continuous measurement of rectal temperature was started. At midnight, subjects went

to bed, and the 1st sleep period (sleep 1) was recorded. The next morning (day 1), subjects were woken up at 7 a.m. Then they had breakfast and took a shower. Until 2 p.m., subjects were free to read or watch videos. Between 2 and 3 p.m., the EEG electrodes were checked, and electrocardiogram (ECG) electrodes were attached. At 3 p.m., the subjects practiced the test battery; 6 min of wake-EEG (3 min eyes open, followed by 3 min eyes closed) and ECG were recorded, and a saliva sample was collected for the determination of melatonin content. Fifteen minutes prior to each test battery (duration: approximately 15 min), subjects had to remain seated upright in their chairs without moving, as the change of position is known to influence hormonal concentrations (Deacon and Arendt, 1994). Warm meals were scheduled at the same time for all subjects, and snacks and beverages were available on request. No consumption was allowed in the 45-min interval prior to the collection of a saliva sample. After each consumption, the subjects had to rinse their mouths with water to prevent contamination of the next saliva sample.

The 1st testing period (testing 1) with hourly measurements started at 6 p.m. and lasted until 5 a.m. the next morning (day 2). During this period of sustained wakefulness, from midnight until 4 a.m., the subjects were exposed to 1 of 3 light conditions (see below). From 6 a.m. to 2 p.m. (day 2), the 2nd sleep period (sleep 2) was recorded. At 2 p.m. (day 2), subjects were woken up again; they had breakfast and took a shower, and the 2nd testing period (testing 2) started at 6 p.m., which lasted until 2 a.m. the next day (day 3). From 2 a.m. onwards, the 3rd and last sleep period (sleep 3) was recorded. Spontaneous sleep termination was recorded (i.e., subjects were instructed to sleep as long as they wanted and to give a sign via the intercom when they felt refreshed and wanted to get up).

### Light Exposure

The light intensity was < 10 lux during the whole experiment, except for the period of light exposure (midnight until 4 a.m. on day 2) and the sleeping periods (lights off = 0 lux). During light exposure, subjects sat in comfortable chairs with headrests in front of a video monitor at a distance of 5 m and watched videos, thus focusing their eyes on the middle of the TV screen. Two light boxes (Bright Light; Philips, Eindhoven; the Netherlands, with TL tubes PLL55W) were placed at the left and right relative to the direc-

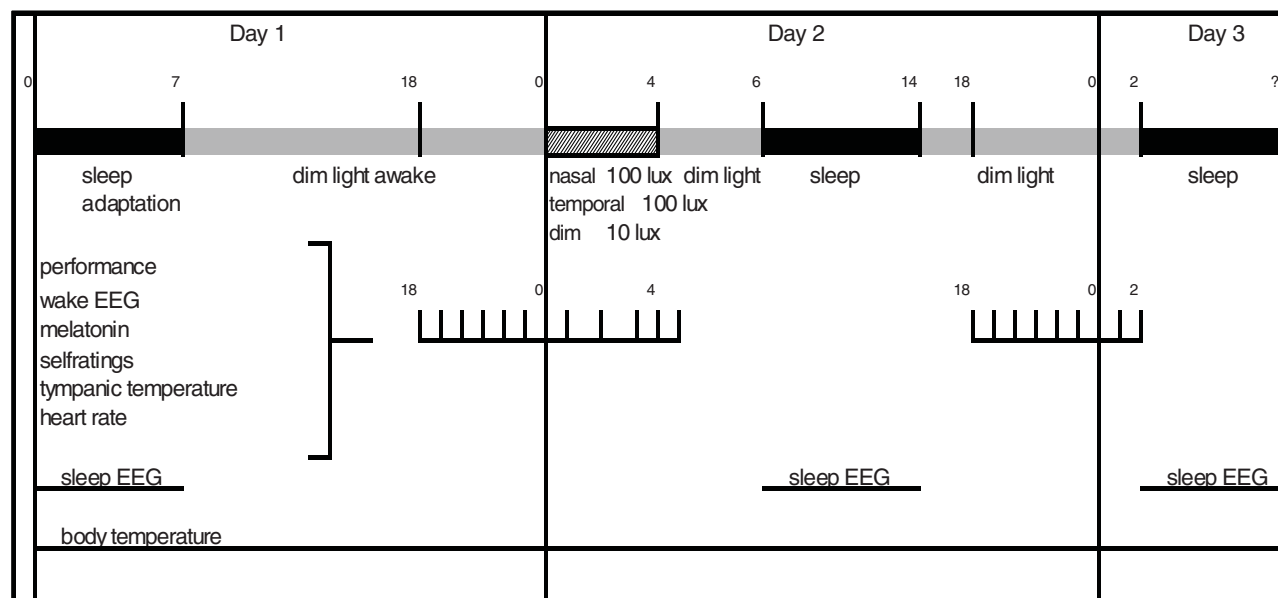


Figure 1. Timetable of the experiment. Black bars indicate the sleeping periods, gray bars the period of sustained wakefulness in dim light (< 10 lux), and hatched bars the period of light exposure during the whole experiment. The testing periods with hourly measurements (all measured variables are listed before the bracket) are indicated by black lines with tick marks on them. Continuous measurements (sleep EEG and core body temperature) are indicated by black lines without tick marks.

tion of gaze of the subjects, at a distance of 100 cm from the eye. Parts of the light boxes were covered to leave a vertical rectangular field of illumination. With respect to the direction of gaze, the field of illumination ranges horizontally from 17.6 to 30 degrees and vertically from  $-15.4$  to  $+15.4$  degrees. All subjects could see the light source entirely with both eyes, which certifies that the 2 light sources illuminated equal areas of the retina.

Subjects were then exposed individually to 100 lux of temporal illumination, 100 lux of nasal illumination, or dim light (control condition; light intensity below 10 lux). Total irradiance of the 100-lux pulse was  $340 \mu\text{W}/\text{cm}^2$ , with a photon density of  $9.4 \times 10^{13}$  photons/sec/cm<sup>2</sup>. To expose either the temporal or the nasal part of the retina exclusively, subjects wore individual adjustable helmets with black shields attached. In the temporal condition, shields of black cardboard were attached to the left and right sides of the helmet, ensuring that light from the left Bright Light box only entered the right eye of the subject and vice versa. For the nasal condition, the shield was placed between the eyes and above and along the nose, so that the light from the left lamp only entered the left eye and the light from the right lamp only entered the right eye (for details, see Visser et al.,

1999). In the dim-light condition, subjects wore the helmets without shields. Great care was taken to position the light sources at exact equal distances from the eyes (100 cm) in all conditions.

### Pupil Dilation

To minimize the effect of variations in pupil size (Gaddy et al., 1993), 1.5 h before light exposure (at 10:30 p.m.), 1 droplet of 1% cyclopentolate hydrochloride (Bournonville Pharma, the Hague, the Netherlands) was administered to both eyes of the subjects. The treatment was repeated at 11:30 p.m. In addition to pupil dilation, cyclopentolate rendered it impossible for the subjects to accommodate their eyes. The corresponding visual problems were compensated with adequate spectacles. The problems were over within 24 h.

### Core Body Temperature

Core body temperature was measured continuously online with the PUCK Temperature Telemetry system (Ambulatory Monitoring, Ardsley, NY), consisting of an ambulant rectal thermometer, a transmit-

ter, and a receiver. Data were stored at 1-min intervals. Due to sanitary requirements and technical problems, there were missing data that were linearly interpolated. In total, this concerned 4% of the CBT data. Furthermore, we replaced 2 h of the signal through linear interpolation each time the subjects had a shower (twice during the whole experiment, on days 1 and 2).

To assess the light-induced phase shift, a running average of 1 h was calculated for the period from 9 p.m. of the 1st evening until 6 a.m. of the 3rd day. To reduce the sensitivity to noise fluctuations, the maximum, minimum, and midrange of CBT values were calculated after leaving out the 10% highest and lowest values. The crossing points of the CBT curves with the midrange were defined as "rise" and "drop" points and served as circadian markers. The possible phase shifts in CBT on the 3rd day were corrected for the circadian phase of the 1st day and were tested with repeated-measures ANOVA.

Immediate effects of the different light conditions on CBT were tested with repeated-measures ANOVA for the factors of condition (nasal, temporal, and dim) and exposure (before: 9, 10, 11, and 12 p.m. vs. during: 1, 2, 3, and 4 a.m.). The interaction effect of condition and exposure will be presented.

## Melatonin

Melatonin concentrations were measured in saliva. Saliva samples were taken every hour prior to the test battery. Saliva was collected using Sarstedt Salivettes (Sarstedt BV, Etten-Leur, the Netherlands) with a polyester swab. Samples were centrifuged immediately and stored at  $-20^{\circ}\text{C}$ . Melatonin concentration was determined by means of a radioimmunoassay (RIA; rabbit antibody supplied by Stockgrand Ltd., Guildford, Surrey, UK; SAC-Cel anti-rabbit by Lucron Bioproducts, Gennep, the Netherlands; and 2-[125] Iodomelatonin by Amersham Biosciences, Roosendaal, the Netherlands).

The limit of detection for the RIA was 0.39 pg/mL with an intra-assay variation of 11% at a low melatonin concentration ( $\bar{x} = 5.02$  pg/mL,  $n = 15$ ) and 9% at a high melatonin concentration ( $\bar{x} = 88.50$  pg/mL,  $n = 15$ ). Interassay covariance was 16.79% at a low melatonin concentration ( $\bar{x} = 4.41$  pg/mL,  $n = 18$ ) and 13.11% at a high melatonin concentration ( $\bar{x} = 73.44$  pg/mL,  $n = 18$ ).

DLMO was used as a phase marker and was calculated per individual per condition. DLMO was

defined as the time at which the melatonin profile of an individual crossed a threshold of 25% of the maximum value reached during the 1st night of the dim-light condition. If this relative threshold value was below 5 pg/mL, an absolute value of 5 pg/mL was used to avoid approaching the lower detection limit. DLMO was determined by linear interpolation between the last sample with a lower concentration and the 1st sample with a higher concentration than the threshold value. The shift in DLMO on the 2nd night relative to the 1st night of the temporal and nasal conditions was tested against the shift in the dim-light condition using repeated-measures ANOVA with the factors of night and condition and included 11 subjects. Immediate effects on melatonin concentration were tested with repeated-measures ANOVA for the factors of condition (nasal, temporal, and dim) and exposure (before: 9, 10, 11, and 12 p.m. vs. during: 1, 2, 3, and 4 a.m.), as well as their interaction.

## Sleepiness

The test battery that subjects had to complete once per hour on a PC consisted of questionnaires and performance tests. Questionnaires included the Visual Analogue Scale for Fatigue (VAS-*f*; Lee et al., 1990) and the Karolinska Sleepiness Scale (KSS; Åkerstedt and Gillberg, 1990) to assess subjective sleepiness. Immediate changes in sleepiness were tested for the period before exposure (9, 10, 11, and 12 p.m.) versus the period during light exposure (1, 2, 3, and 4 a.m.) by repeated-measures ANOVA. To test for possible phase shifts in the rhythm of sleepiness, "sleepiness onset" was determined per individual per condition. The threshold we used as a criterion for sleepiness onset was the minimum score plus 25% of the difference between the minimum and the maximum in the dim-light condition per person. The sleepiness onset time points were determined by linear interpolation between the last sleepiness score with a lower value and the 1st sleepiness score with a higher value than the individual criterion.

## RESULTS

### Immediate Effects

Figure 2 (upper panel) shows that the circadian drop in CBT during the 4 h of nasal and temporal illu-

mination is not different from the circadian drop of CBT in the control condition. ANOVA with repeated measures revealed no significant differences between conditions,  $F(2, 10) = 0.122$ ,  $p > 0.1$ .

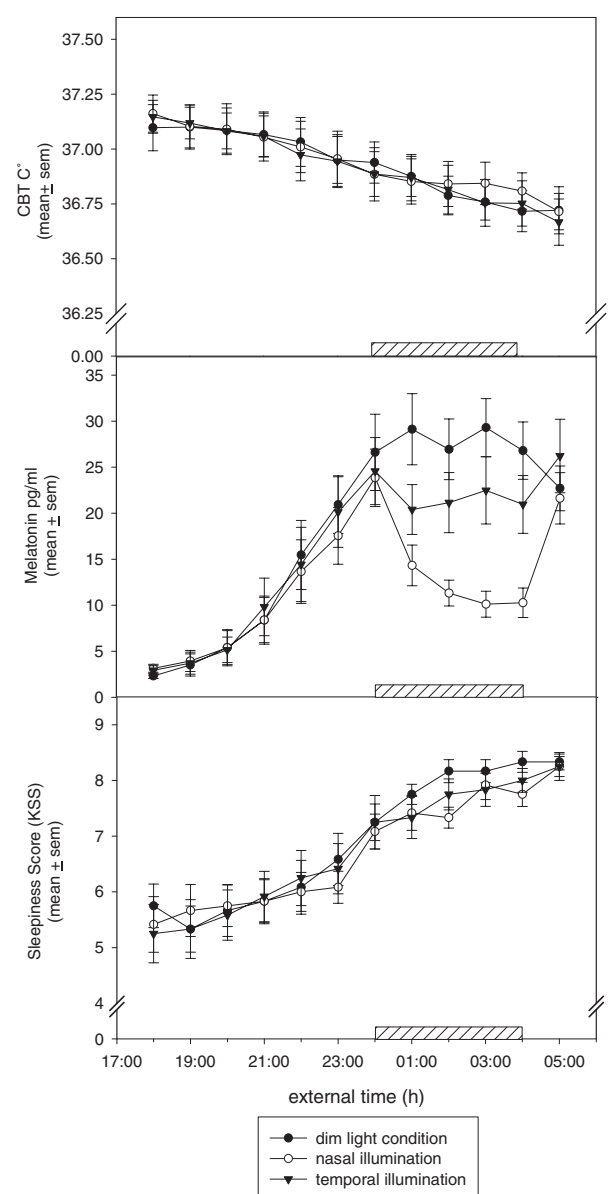
The middle panel of Figure 2 shows a clear suppression of melatonin concentration during both experimental conditions compared to the dim-light condition. The same statistical approach as for the CBT analyses was used for the melatonin concentration. The ANOVA revealed a significant effect of condition,  $F(2, 10) = 12.27$ ,  $p = 0.002$ . A further ANOVA for repeated measurements revealed a significant suppression of melatonin in the nasal relative to the dim-light condition,  $F(1, 11) = 34.681$ ,  $p = 0.000$ , as well as for the temporal versus the dim-light condition,  $F(1, 11) = 12.272$ ,  $p = 0.005$ . There was also a significant difference between the temporal and the nasal conditions, with the greatest suppression of melatonin found in the nasal light condition,  $F(1, 11) = 22.369$ ,  $p = 0.001$ .

Immediate effects of the different light conditions on subjective sleepiness were also tested using an ANOVA with repeated measurements. As can be seen in Figure 2 (lower panel), levels of sleepiness rose continuously over the whole period of 23 h of sustained wakefulness. ANOVA with repeated measures showed no significant differences between the conditions,  $F(2, 10) = 0.723$ ,  $p > 0.5$ .

### Phase-Shifting Effects

In Figure 3, the course of CBT is shown, using a 1-h running average for the period from 9 p.m. on day 0 to 6 a.m. on day 3. The rise and drop points of CBT are marked. Mean rise and drop times are listed in Table 1. The standard deviations on day 1 indicate that subjects varied considerably in their circadian phase on day 1. Therefore, we calculated the shift in response to the different light conditions relative to the phase on day 1. Concerning the rise of CBT, the shifts were  $+30 \pm 100$  min (dim-light condition),  $+46 \pm 97$  min (nasal light condition), and  $-41 \pm 89$  min (temporal light condition). For the drop of CBT, the shifts were  $0 \pm 133$  min (dim-light condition),  $-55 \pm 98$  min (nasal condition), and  $-73 \pm 83$  min (temporal condition). Repeated-measures ANOVA showed neither a significant difference for the shift in the rise points,  $F(2, 7) = 1.864$ ,  $p > 0.1$ , nor a significant difference for the shift in the drop points of CBT,  $F(2, 5) = 0.500$ ,  $p > 0.5$ .

Figure 4 (upper panels) shows the melatonin profile and DLMOs of the 1st night (panel A) and 2nd



**Figure 2.** The course of core body temperature (CBT; upper panel), melatonin concentration (middle panel), and subjective sleepiness (lower panel) before, during, and after the light exposure. The hatched bar indicates the period of light exposure (midnight until 4 a.m.). Repeated-measures ANOVA for the 4 h before (9, 10, 11, and 12 p.m.) versus the 4 h during (1, 2, 3, and 4 a.m.) light exposure revealed no significant condition effect of the different light conditions on CBT ( $p > 0.1$ ) and sleepiness ( $p > 0.5$ ) but did reveal a significant condition effect on melatonin concentration ( $p = 0.002$ ).

night (panel B) for those 11 subjects for whom DLMO on the 1st night could be calculated in all 3 conditions. Average DLMO times are also listed in Table 1.

The overall repeated-measures ANOVA comparing all 3 conditions in a single test revealed a trend for

Table 1. Average rise and drop times of CBT on the 1st and the 2nd days and average DLMO times for the 1st and 2nd nights, including change scores from the 1st to 2nd nights ( $n = 11$ ) ( $\bar{x} \pm SD$ )

	DLMO			CBT			
	1st Night	2nd Night	Difference between 1st and 2nd Nights	1st Day		2nd Day	
				Rise Times	Drop Times	Rise Times	Drop Times
Dim light							
Time	9:32 p.m.	10:10 p.m.	-38 min	10:41 a.m.	11:44 p.m.	10:11 a.m.	11:44 a.m.
SD	52 min	74 min	37 min	135 min	126 min	77 min	76 min
Nasal							
Time	9:45 p.m.	11:12 p.m.	-78 min	11:37 a.m.	00:08 a.m.	10:52 a.m.	1:04 a.m.
SD	70 min	85 min	28 min	121 min	113 min	142 min	62 min
Temporal							
Time	9:41 p.m.	10:44 p.m.	-63 min	10:20 a.m.	11:06 p.m.	11:03 a.m.	00:19 a.m.
SD	69 min	87 min	29 min	104 min	119 min	67 min	115 min

NOTE: DLMO = dim-light melatonin onset; CBT = core body temperature.

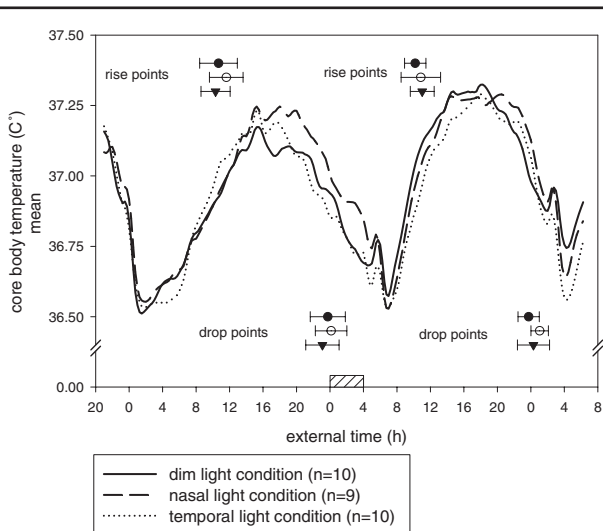


Figure 3. The course of core body temperature (CBT) for the whole period of the experiment. The line graphs represent a 1-h running average for each group (dim-light condition,  $n = 10$ ; nasal condition,  $n = 9$ ; and temporal condition,  $n = 10$ ), plus "rise" and "drop" (mean  $\pm$  SEM) points of CBT for the first day (left rise and drop points) and the third day (right rise and drop points). Dim rise and drop points are indicated by filled circles, nasal rise and drop points are indicated by open circles, and temporal rise and drop points are indicated by triangles. The hatched bar indicates the period of light exposure. Repeated-measures ANOVA showed neither a significant difference between the shift of the rise points ( $p > 0.1$ ) nor the shift of the drop points of CBT ( $p > 0.5$ ).

the Condition  $\times$  Night interaction,  $F(2, 9) = 3.772$ ,  $p = 0.065$ . Tested with a repeated-measures ANOVA, we found a significant delay of DLMO in the nasal condition ( $-78 \pm 29$  min) compared to the dim-light condition ( $-38 \pm 37$  min),  $F(1, 10) = 8.277$ ,  $p = 0.016$ . There

was a trend for the delay of DLMO in the temporal condition ( $-63 \pm 28$  min) compared to the dim-light condition,  $F(1, 10) = 3.889$ ,  $p = 0.077$ . There was no significant effect for comparison with the nasal condition,  $F(1, 10) = 2.851$ ,  $p = 0.122$ .

The lower panels of Figure 4 show the course of sleepiness for the 1st night (panel C) and the 2nd night (panel D). Sleepiness onset could only be calculated for the 2nd night because for the 1st night, less than 50% of the subjects met the chosen criterion because they lacked a clear rise in sleepiness. The onset time points for each condition are marked, including the maximal number of subjects per condition. Due to fluctuations in sleepiness scores, onset times could not always be determined unequivocally. This hampered comparisons between conditions. Since complete sets of onset times were available for 7 subjects, we only compared conditions pairwise. We found no significant differences in the rhythm of sleepiness when comparing the following: the nasal to the dim-light condition,  $t(1, 7) = -0.277$ ,  $p > 0.5$ ; the temporal to the dim-light condition,  $t(1, 9) = 0.025$ ,  $p > 0.5$ ; or the nasal to the temporal condition,  $t(1, 6) = 0.412$ ,  $p = 0.5$ . To explore whether the same photoreceptors are responsible for immediate and phase-shifting effects, we calculated the correlation between melatonin suppression and phase shift (Fig. 5). The amount of melatonin suppression in the experimental conditions is expressed as the mean of the light exposure period (1-4 a.m.) relative to dim light and is correlated (Pearson) with the size of the phase shift on the 2nd day relative to the first. We found a positive correlation between the amount of suppression and the size of the phase



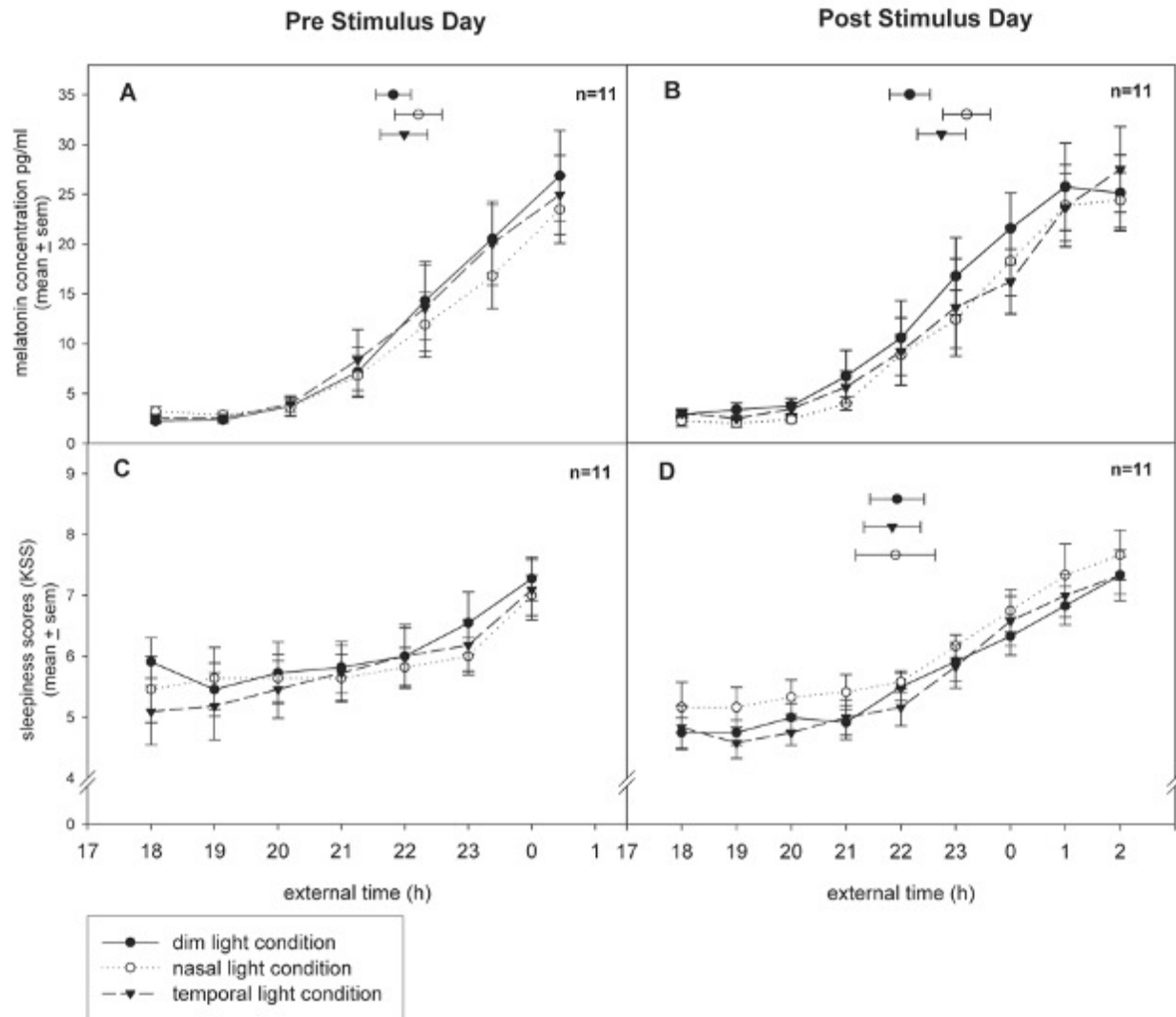


Figure 4. The mean courses ( $n = 11$ ) of melatonin concentration and subjective sleepiness for the prestimulus day (panels A and C) and the poststimulus day (panels B and D), including DLMO rise and sleepiness onsets times ( $\bar{x} \pm S_{\bar{x}}$ ). A repeated-measures ANOVA showed a significant delay of DLMO in the nasal condition ( $p = 0.016$ ) and a trend in the temporal condition ( $p = 0.077$ ) when compared to the shift in the dim-light condition. Average sleepiness onset times are depicted for the maximal number of subjects per condition (dim light = 12, nasal = 8, temporal = 10) and are tested with a paired  $t$  test, revealing no significant differences in sleepiness onsets among the following conditions: nasal versus dim light,  $t(1, 7) = -0.277, p > 0.5$ ; temporal versus dim light,  $t(1, 9) = 0.025, p > 0.5$ ; and nasal versus temporal,  $t(1, 6) = 0.412, p = 0.5$ .

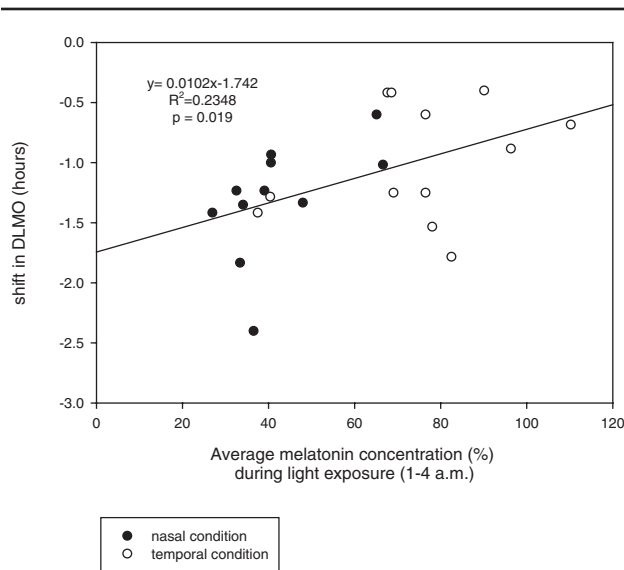
shift ( $r = 0.486, p = 0.019$ ); that is, more suppression of melatonin resulted in larger phase shifts.

## DISCUSSION

The aim of our study was to investigate possible functional differences between the photoreceptors in the nasal and the temporal part of the human retina. This might be helpful in suggesting whether the same circadian photoreceptors that mediate the melatonin

suppression in humans are also responsible for phase shifts. For this reason, we extended the design of the Visser et al. (1999) study to a 2.5-day protocol, which enabled us to study at least 1st-cycle phase shifts. Furthermore, we added the measurement of psychological and physiological variables (subjective sleepiness and wake/sleep EEG) to the protocol, and we dilated the subjects' pupils to exclude interference of pupillary responses.

Regarding the immediate effects, the data show that 4 h of 100 lux of nasal illumination of the retina led



**Figure 5.** The correlation of the average melatonin concentration (during the light period) in the nasal and temporal conditions relative to the dim-light condition, as well as the shift of melatonin.

to significantly greater melatonin suppression than temporal illumination, despite equal size of exposed areas. Using 2 h of 500 lux (undilated pupils), Visser et al. (1999) found a melatonin suppression of 33% in the nasal condition at the end of their light period (3:30) relative to the average of the prestimulus melatonin concentration values at 00:45 and 1:30 a.m. In the present study, we found a melatonin suppression of 39% in the nasal condition at the end of the light period at 4 a.m. (dilated pupils) relative to melatonin concentration at 11 and 12 p.m. Since Visser et al. (1999) did not have a dim-light control condition, we can mainly compare final melatonin concentration with nasal stimulation relative to temporal stimulation. The ratio amounts to 0.54 for Visser et al. (1999) versus 0.59 in the present study. The similarity of the results demonstrates that the different responses of the nasal and temporal retinal areas are not due to differences between conditions in pupillary constriction. Although most studies mention local differences in the density of light-sensitive retinal ganglion cells across the retina (Provencio et al., 2000; Berson et al., 2002; Hattar et al., 2002; Hannibal et al., 2002), only the study by Hannibal et al. (2002) provides a quantitative estimate of the differences across the retina, which was quantified for the rat. The concentration of melanopsin-containing retinal ganglion cells is reported to be 4- to 5-fold higher in the superior half of the rat retina. The orientation of the borderline

between superior and inferior halves of the retina has not been specified by Hannibal et al. (2002). Glickman et al. (2003) compared melatonin suppression upon light exposure of superior and inferior parts of the human retina. They showed larger suppression of melatonin upon exposure of the inferior retina. Combining our data with those collected by Glickman et al. (2003), we must conclude that in humans, 1) substantial differences in the density of light-sensitive retinal ganglion cells occur across the retina, and 2) a significant gradient in density is present both in the horizontal and vertical directions. The highest density of these retinal ganglion cells occurs in the inferior nasal area of the retina. Studies to test these differences at the anatomical level remain to be performed.

The suppression of melatonin was not accompanied by a reduction of subjective sleepiness or a reduction of the circadian drop in CBT. These findings are in contrast with earlier results on full-retinal bright-light exposure (Badia et al., 1991; Cajochen et al., 2000; Ruger et al., 2003), in which a reduction of sleepiness and a reduction of the circadian drop of CBT were associated with the suppression of melatonin. On the basis of our current results, we have to conclude that 100 lux of nasal and temporal illumination of the human retina is strong enough to significantly suppress melatonin but not strong enough to influence CBT or subjective sleepiness significantly. Clearly, the suppression of melatonin does not necessarily result in a reduction of subjective sleepiness, as studies of daytime bright-light exposure indicate (Ruger et al., 2002). Phipps-Nelson et al. (2003) found a reduction of subjective sleepiness and an improved performance by light in the absence of melatonin.

The results on possible phase shifts in melatonin, CBT, and sleepiness are inconsistent. On the one hand, nasal illumination resulted in a delay of the melatonin rhythm by  $-78$  min, which falls into the range of delays obtained by full-retinal exposure to bright light during the night (Kubota et al., 2002; Ruger et al., 2003). On the other hand, we found no phase shift of the CBT rhythm in the nasal condition. The discrepancies between the shifts in melatonin and CBT might be due to differences in inherent fluctuations (Klerman et al., 2002). CBT is much more sensitive to masking influences than melatonin. Finally, the course of subjective sleepiness was not delayed by partial illumination of the human retina. This result is in accordance with earlier findings that 4 h of whole-retinal bright-light exposure did not shift the course of sleepiness (Ruger et al., 2003). It is clear from our data that sleepi-

ness scores show too much intraindividual variation, thereby masking genuine circadian phase shifts. A further explanation for the failure to show shifts in CBT and sleepiness rhythms might be our protocol. We did not use a constant-routine or constant-posture protocol as in other studies (Lockley et al., 2003; Warman et al., 2003); that is, the movements of our subjects were limited but not totally restricted, and their food intake was scheduled but not matched in an isocaloric way. Since sleep timing was necessarily different between day 1 and day 2, those masking influences differed also. The calculated phase shifts of CBT and sleepiness are contaminated by masking. For sleepiness, the masking was not due to light exposure or sleep since the rise points were found outside the intervals in which light exposure and sleep were scheduled. For CBT, in all but 1 case, the rise times were found outside the interval of light exposure. Yet rise times and drop times of CBT sometimes occurred during wakefulness and sometimes occurred during sleep. The shifts in body temperature were therefore masked by sleep and wakefulness. However, since the timing of the masking events is identical for all subjects, it should have been possible to see systematic effects of conditions against the backgrounds of the masked signals. A further limitation to our study is the short period of preadaptation. A longer period might have reduced the variation between subjects and created more stable baseline levels of CBT and sleepiness.

Nonetheless, both the suppression of melatonin and the circadian phase shift of the DLMO were greater following nasal illumination. This suggests that they may exploit the same photoreceptors, which appear to be either more numerous or more sensitive in the nasal part of the retina. We correlated the amount of melatonin suppression in the experimental conditions during the light exposure (1-4 a.m.) and the size of phase shift of melatonin and found a significant, positive relation between them ( $r = 0.486$ ,  $p = 0.019$ ); that is, subjects who showed more melatonin suppression had greater phase shifts (Fig. 5). The failure to find further effects on CBT and sleepiness might be due to the fact that the stimulus used was not strong enough or that those variables show larger variability.

#### ACKNOWLEDGMENTS

The authors thank the following people: Elisabeth Pomp and Sander Berends for helping to collect the

data, as well as colleagues of the eye clinic of the Groningen Hospital for generously supervising and providing us with the cyclopentolate. This research was financially supported by the Dutch Technology Foundation, applied science division of Nederlandse Organisatie voor Wetenschappelijk Onderzoek, and the Ministry of Economic Affairs, Philips Domestic Appliances and Personal Care, and Philips Lighting.

#### REFERENCES

- Adler JS, Kripke DF, Loving RT, and Berga SL (1992) Peripheral vision suppression of melatonin. *J Pineal Res* 12:49-52.
- Åkerstedt T and Gillberg M (1990) Subjective and objective sleepiness in the active individual. *Int J Neurosci* 52:29-37.
- Badia P, Myers B, Boecker M, Culpepper J, and Harsh JR (1991) Bright light effects on body temperature, alertness, EEG and behavior. *Physiol Behav* 50:583-588.
- Beersma DG and Daan S (1993) Strong or weak phase resetting by light pulses in humans? *J Biol Rhythms* 8:340-347.
- Berson DM (2003) Strange vision: Ganglion cells as circadian photoreceptors. *Trends Neurosci* 26:314-320.
- Berson DM, Dunn FA, and Takao M (2002) Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295:1070-1073.
- Brainard GC, Hanifin JP, Greeson JM, Byrne B, Glickman G, Gerner E, and Rollag MD (2001a) Action spectrum for melatonin regulation in humans: Evidence for a novel circadian photoreceptor. *J Neurosci* 21:6405-6412.
- Brainard GC, Hanifin JP, Rollag MD, Greeson J, Byrne B, Glickman G, Gerner E, and Sanford B (2001b) Human melatonin regulation is not mediated by the three cone photopic visual system. *J Clin Endocrinol Metab* 86:433-436.
- Cajochen C, Zeitzer JM, Czeisler CA, and Dijk DJ (2000) Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. *Behav Brain Res* 115:75-83.
- Cooper HM, Tessonnaud A, Caldani M, Locatelli A, Richard S, and Viguier Martinez M (1993) Morphology and distribution of retinal ganglion cells (RGC) projecting to the suprachiasmatic nucleus in the sheep. *Am Soc Neurosci Abstr* 19:1704-1704.
- Deacon S and Arendt J (1994) Posture influences melatonin concentrations in plasma and saliva in humans. *Neurosci Lett* 167:191-194.
- French J, Hannon P, and Brainard GC (1990) Effects of bright light illuminance on body temperature and human performance. *Annu Rev Chronopharmacol* 7:37-40.
- Gaddy JR, Edelson M, Stewart K, Brainard GC, and Rollag MD (1992) Possible retinal spatial summation in melatonin suppression. In *Biologic Effects of Light*, Holick MS and Kligman AM, eds, pp 196-204, Berlin, de Gruyter.
- Gaddy JR, Rollag MD, and Brainard GC (1993) Pupil size regulation of threshold of light-induced melatonin suppression. *J Clin Endocrinol Metab* 77:1398-1401.

- Glickman G, Hanifin JP, Rollag MD, Wang J, Cooper H, and Brainard GC (2003) Inferior retinal light exposure is more effective than superior retinal exposure in suppressing melatonin in humans. *J Biol Rhythms* 18:71-79.
- Hannibal J, Hindersson P, Knudsen SM, Georg B, and Fahrenkrug J (2002) The photopigment melanopsin is exclusively present in pituitary adenylate cyclase-activating polypeptide-containing retinal ganglion cells of the retinohypothalamic tract. *J Neurosci* 22:RC191:1-7.
- Hattar S, Liao HW, Takao M, Berson DM, and Yau KW (2002) Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. *Science* 295:1065-1070.
- Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW, Lem J, Biel M, Hofmann F, Foster RG, and Yau KW (2003) Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature* 424:76-81.
- Honma K and Honma S (1988) A human phase response curve for bright light pulses. *Jpn J Psychiatry Neurol* 42:167-168.
- Horne JA and Östberg O (1976) A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *International Journal of Chronobiology* 4:97-110.
- Khalsa SB, Jewett ME, Cajochen C, and Czeisler CA (2003) A phase response curve to single bright light pulses in human subjects. *J Physiol* 549:945-952.
- Klerman EB, Gershengorn HB, Duffy JF, and Kronauer RE (2002) Comparisons of the variability of three markers of the human circadian pacemaker. *J Biol Rhythms* 17:181-193.
- Kubota T, Uchiyama M, Suzuki H, Shibui K, Kim K, Tan X, Tagaya H, Okawa M, and Inoue S (2002) Effects of nocturnal bright light on saliva melatonin, core body temperature and sleep propensity rhythms in human subjects. *Neurosci Res* 42:115-122.
- Lasko TA, Kripke DF, and Elliot JA (1999) Melatonin suppression by illumination of upper and lower visual fields. *J Biol Rhythms* 14:122-125.
- Lee KA, Hicks G, and Nino-Murcia G (1990) Validity and reliability of a scale to assess fatigue. *Psychiatry Res* 36:291-298.
- Lockley SW, Brainard GC, and Czeisler CA (2003) High sensitivity of the human circadian melatonin rhythm to resetting by short wavelength light. *J Clin Endocrinol Metab* 88:4502-4505.
- Lucas RJ, Douglas RH, and Foster RG (2001) Characterization of an ocular photopigment capable of driving pupillary constriction in mice. *Nat Neurosci* 4:621-626.
- Lucas RJ, Freedman MS, Munoz M, Garcia-Fernandez JM, and Foster RG (1999) Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors. *Science* 284:505-507.
- Lucas RJ, Hattar S, Takao M, Berson DM, Foster RG, and Yau KW (2003) Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science* 299:245-247.
- Minors DS, Waterhouse JM, and Wirz-Justice A (1991) A human phase-response curve to light. *Neurosci Lett* 133:36-40.
- Panda S, Sato TK, Castrucci AM, Rollag MD, DeGrip WJ, Hogenesch JB, Provencio I, and Kay SA (2002) Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. *Science* 298:2213-2216.
- Phipps-Nelson J, Redman JR, Dijk DJ, and Rajaratnam SM (2003) Daytime exposure to bright light, as compared to dim light, decreases sleepiness and improves psychomotor vigilance performance. *Sleep* 26:695-700.
- Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, and Rollag MD (2000) A novel human opsin in the inner retina. *J Neurosci* 20:600-605.
- Ruby NF, Brennan TJ, Xie X, Cao V, Franken P, Heller HC, and O'Hara BF (2002) Role of melanopsin in circadian responses to light. *Science* 298:2211-2213.
- Rüger M, Gordijn MC, Beersma DG, de Vries B, and Daan S (2002) Bright light effects at night and day on various measures of sleepiness. In *Sleep-Wake Research in the Netherlands*, Van Bommel AL, Beersma DG, Hofman WF, Ruit GSF, and Vos PJE, eds, pp 95-99, Alphen aan den Rijn, the Netherlands, Van Zuiden Communications BV.
- Rüger M, Gordijn MC, Beersma DG, de Vries B, and Daan S (2003) Acute and phase-shifting effects of ocular and extraocular light in human circadian physiology. *J Biol Rhythms* 18:409-419.
- Thapan K, Arendt J, and Skene DJ (2001) An action spectrum for melatonin suppression: Evidence for a novel non-rod, non-cone photoreceptor system in humans. *J Physiol* 535:261-267.
- Visser EK, Beersma DG, and Daan S (1999) Melatonin suppression by light in humans is maximal when the nasal part of the retina is illuminated. *J Biol Rhythms* 14:116-121.
- Warman VL, Dijk DJ, Warman GR, Arendt J, and Skene DJ (2003) Phase advancing human circadian rhythms with short wavelength light. *Neurosci Lett* 342:37-40.