Lighting up the clock
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Chapter 6

Nasal versus temporal illumination of the human retina: effects on core body temperature, melatonin, and circadian phase

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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. We here 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 three 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 three 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. 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.
Introduction

The mammalian eye contains a third 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 (Provencio et al., 2000; Hattar et al., 2002). 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 suprachiasmatic nucleus (SCN), the ganglion cells are involved in the entrainment of the circadian system to the light-dark cycle (Panda et al., 2002; Ruby et al., 2002; Hattar et al., 2003). They also contribute to the pupillary light reflex (Lucas et al., 2001; Lucas et al., 2003; Hattar et al., 2003), and they 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 (French et al., 1990; Badia et al., 1991; 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 446-477 nm (peak wavelength 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 non-rod, non-cone photoreceptor. The action spectrum that fitted the data best followed a rhodopsin template with a wavelength of $\lambda_{\text{max}} = 459$ 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. (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. (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 ($4300 \, \mu\text{W/cm}^2$) than the short wavelength light ($28 \, \mu\text{W/cm}^2$). Gaddy et al. (Gaddy et al., 1992; Gaddy et al., 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. (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. (Visser et al., 1999) showed that illumination of the nasal part of the human retina by a 4-hrs stimulus of 500 lux yielded a larger suppression of salivary melatonin than illumination of the temporal part, irrespective of whether the upper or lower part of the retina were illuminated. Lasko et al. (Lasko et al., 1999) compared salivary melatonin suppression in response to either 500 lux in the upper or in the lower visual field for three hours. The largest suppression of melatonin was obtained by the illumination of the upper visual field (the lower half of the retina). Glickman and colleagues (Glickman et al., 2003) explored the changes in plasma melatonin levels due to 90-min 200 lux illumination of either the superior retina, the inferior retina, or 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 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 one design. The timing of the light exposure was based on human phase response curves on ocular light pulses and chosen in such a way that maximal delays were expected (Honma and Honma, 1988; Minors et al., 1991; Beersma and Daan, 1993; Khalsa et al., 2003)
Methods

Subjects

A total of 7 female and 5 male healthy subjects (mean age: 21, SD ± 2.2 years) participated in the study. Subjects were screened by using a general health questionnaire and a Morningness-Eveningness-Questionnaire (Horne and Östberg, 1976). Only non-smoking, non-extreme 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 had to be without a history of psychiatric illness and had not 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 contraceptive were tested during the phase they took a contraceptive with a stable hormone concentration.

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

Time Isolation Facility

The protocol included three stays of 2,5 days each in the time isolation facility. The facility, where neither daylight nor clock information are present, can host four subjects simultaneously in separate rooms. Subjects could read or study, listen to music, watch videos, or perform other non-physical 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 till 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 electro-encephalogram (EEG) (2 channels C3-A2; Fz-A1), electro-oculogram (EOG), and electromyogram (EMG). At the same time the test battery was introduced and explained and continuous measurement of rectal temperature started. At midnight subjects went to bed and the first 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 ECG electrodes were attached. At 3 p.m. the subjects practiced the test battery and six minutes 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 chair 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, snacks and beverages were available on request. No consumptions were allowed in the 45 minutes interval prior to the collection of a saliva sample. After each consumption the subjects had to rinse their mouth with water to prevent contamination of the next saliva sample.

![Figure 1](image_url)

Figure 1. Timetable of the experiment, black bars indicate the sleeping periods, grey bars the period of sustained wakefulness in dim light (< 10 lux), and white 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.

The first testing period (Testing 1) with hourly measurements started at 6 p.m. and lasted till 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 one of three light conditions (see below). From 6 a.m. till 2 p.m. (Day 2) the second 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 second testing period (Testing 2) started which lasted till 2 a.m. of the next day (Day 3). From 2 a.m. onwards the third 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 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 were seated in a comfortable chair with headrest in front of a video monitor at a distance of 5 m and watched videos, thus retaining their eyes oriented towards the middle of the TV screen. Two light boxes (Bright Light\textsuperscript{®}, Philips, Eindhoven, The Netherlands, with TL tubes PLL55W) were placed at the left and at the right relative to the direction 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 of their eyes, which certifies that the two light sources illuminated equal areas of the retina.

Subjects were then exposed individually either 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 340µW/cm\textsuperscript{2} with a photon density of 9.4 x 10\textsuperscript{13} photons/sec/cm\textsuperscript{2}. In order 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 side of the helmet ensuring that light of the left Bright Light\textsuperscript{®} 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 of the left lamp entered the left eye and the light of 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 exactly equal distance from the eyes (100 cm) in all conditions.
**Pupil dilation**

To minimize the effect of variations in pupil size (Gaddy et al., 1993) one and a half hour before light exposure (at 10:30 p.m.) one 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 (CBT) was measured continuously online with the PUCK Temperature Telemetry system (Ambulatory Monitoring, Inc., Ardsley, NY, U.S.A.), consisting of an ambulant rectal thermometer, a transmitter, 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 two hours of the signal through linear interpolation each time the subjects had a shower (twice during the whole experiment; on **Day 1** and on **Day 2**).

To assess the light induced phase shift, a running average of one hour was calculated for the period from 9 p.m. of the first evening until 6 a.m. of the third 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’ points and ‘drop’ points and served as circadian markers. The possible phase shifts in CBT on the third day were corrected for the circadian phase of the first day and tested with repeated measures ANOVA.

Immediate effects of the different light conditions on CBT were tested with repeated measurement ANOVA for the factors condition (nasal, temporal, and dim), 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°C. Melatonin concentration was determined by means of a RIA immunoassay (Rabbit antibody supplied by Stockgrand Ltd., Guildford Surrey, UK; SAC-Cel anti-Rabbit by Lucron Bioproducts, Gennep, The Netherlands; 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 (mean 5.02 pg/ml, n = 15) and 9% at a high melatonin concentration (mean 88.50 pg/ml, n = 15). Inter-assay covariance was 16.79% at a low melatonin concentration (mean 4.41 pg/ml, n = 18) and 13.11% at a high concentration (mean 73.44 pg/ml, n = 18).

Dim Light Melatonin Onset (DLMO) was used as a phase marker and 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 first 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, in order to avoid approaching the lower detection limit. DLMO was determined by linear interpolation between the last sample with a lower and the first sample with a higher concentration than the threshold value. The shift in DLMO on the second night relative to the first night of the temporal and nasal condition was tested against the shift in the dim light condition using repeated measures ANOVA with the factors night and condition and included eleven subjects. Immediate effects on melatonin concentration were tested with repeated measurement ANOVA for the factors condition (nasal, temporal, and dim), exposure (before: 9, 10, 11, and 12 p.m. vs. during: 1, 2, 3, and 4 a.m.) and 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., 1991) 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.) vs. during (1, 2, 3, and 4 a.m.) and their interaction.
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 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 and the first sleepiness score with a higher value than the individual criterion.

**Results**

**Immediate effects**

Figure 2 (upper panel) shows that the circadian drop in core body temperature (CBT) during the 4 hours of *nasal* and *temporal* illumination 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 condition 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 hours of sustained wakefulness. ANOVA with repeated measures showed no significant differences between the conditions (F (2,10) = 0.723, p > 0.5).

**Figure 2.** The course of core body temperature (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 till 4 a.m.). Repeated measures ANOVA for the four hours before (9, 10, 11, and 12 pm.) against the four hours 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 on melatonin concentration (p = 0.002).
Nasal versus temporal retinal illumination

Melatonin pg/ml (mean ± sem)

CBT °C (mean ± sem)

Sleepiness Score (KSS) (mean ± sem)

- dim light condition
- nasal illumination
- temporal illumination

External time (h)

17:00  19:00  21:00  23:00  01:00  03:00  05:00
**Phase-shifting effects**

In Figure 3 the course of CBT is shown, using a 1-h running average for the period of 9 p.m. on day 0 until 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 of $-41 \pm 89$ min. (temporal condition). For the drop of CBT the shifts were $0 \pm 133$ min (dim 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 of the drop points of core body temperature ($F (2,5) = 0.500, p > 0.5$).

![Figure 3](image-url)

Figure 3. The course of core body temperature (CBT) for the whole period of the experiment. The line graphs represent a 1-hour running average for each group (dim condition: $n = 10$, nasal condition: $n = 9$, and temporal condition: $n = 10$), plus “rise” and “drop” (mean ± sem) points of CBT for the first (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 open circles, and temporal rise and drop points are 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 core body temperature ($p > 0.5$).
Table 1. Average Rise and Drop times of CBT on the first and the second day (left part) and average DLMO times for the first and the second night, including change scores from the first to the second night, (n = 11) (mean ± sd).

<table>
<thead>
<tr>
<th></th>
<th>DLMO</th>
<th>CORE BODY TEMPERATURE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1(^{st}) Night</td>
<td>2(^{nd}) Night</td>
</tr>
<tr>
<td></td>
<td>DLMO times</td>
<td>DLMO times</td>
</tr>
<tr>
<td>Dim</td>
<td>21:32 p.m. sd ± 52 min</td>
<td>22:10 p.m. sd ± 74 min</td>
</tr>
<tr>
<td>Nasal</td>
<td>21:45 p.m. sd ± 70 min</td>
<td>23:12 p.m. sd ± 85 min</td>
</tr>
<tr>
<td>Temporal</td>
<td>21:41 p.m. sd ± 69 min</td>
<td>22:44 p.m. sd ± 87 min</td>
</tr>
</tbody>
</table>

Figure 4 (upper panels) shows the melatonin profile and DLMOs of the first (panel A) and the second night (panel B) for those eleven subjects of whom DLMO on the first night could be calculated in all three conditions. Average DLMO times are also listed in Table 1. The overall repeated measurements ANOVA comparing all three conditions in a single test revealed a trend for the interaction between condition x night (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 ± 29 min) compared to the dim condition (-38 ± 37 min) (F (1,10) = 8.277, p = 0.016). There was a trend for the delay of DLMO in the temporal condition (-63 ± 28 min) compared to the dim light condition (F (1,10) = 3.889, p = 0.077) and no significant effect for the 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 first night (panel C) and the second night (panel D). “Sleepiness onset” could only be calculated for the second night, because for the first 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 unequivocally be determined. 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 nasal to the dim condition (t (1,7) = -0.277, p > 0.5), the temporal to the dim 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 (Figure 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 correlated (Pearson) with the size of the phase shift on the second day relative to the first. We found a positive correlation between the amount of suppression and size of phase shift (r = 0.486, p = 0.019), i.e. more suppression of melatonin resulted in larger phase shifts.

![Graphs showing changes in melatonin concentration and sleepiness scores over time for different light conditions.](image)
Figure 4. The mean courses (n = 11) of melatonin concentration and subjective sleepiness for the pre stimulus (panel A and C) and the post stimulus day (panel B and D), including DLMO rise and sleepiness onset times (mean ± sem). 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 = 12, nasal = 8, temporal = 10) and tested with a paired t-test, revealing no significant differences in sleepiness onsets between conditions (nasal vs. dim: t(1,7) = -0.277, p >0.5, temporal vs. dim t(1,9) = 0.025, p > 0.5, and nasal vs. temporal: t(1,6) = 0.412, p = 0.5).

Figure 5. The correlation of the average melatonin concentration (during the light period) in the nasal and temporal condition relative to dim, and the shift of melatonin.
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 first 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 to significantly greater melatonin suppression than temporal illumination, despite equal size of exposed areas. Using 2 hours 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 (03:30) 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. In the lack of a dim light control condition in the study by Visser et al. (1999), we can mainly compare final melatonin concentration upon 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 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 paper by Hannibal and colleagues 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) that a significant gradient in density is
present both in the horizontal and in the vertical direction. 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 and/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; Rüger et al., 2003), where a reduction of sleepiness and a reduction of the circadian drop of CBT were associated with the suppression of melatonin. Based on our current results we have to conclude that 100 lux of nasal and temporal illumination of the human retina are 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 (Rüger et al., 2002). Phipps-Nelson and co-workers (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; Rüger 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 core body temperature 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 (Rüger et al., 2003). It is clear from our data that sleepiness scores show too much intra-individual variation, thereby masking genuine circadian phase shifts. A further explanation for the failure to show shifts in core body temperature 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), i.e. 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 core body temperature 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 core body temperature, in all but one case, the rise times were found outside the interval of light exposure. Yet rise times and drop times of core body temperature sometimes occurred during wakefulness and sometimes they 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 pre-adaptation. A longer period might have reduced the variation between subjects and created more stable baseline levels of core body temperature 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 ($r = 0.486, p = 0.019$), \textit{i.e.} subjects that showed more melatonin suppression had greater phase shifts (Figure 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.

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