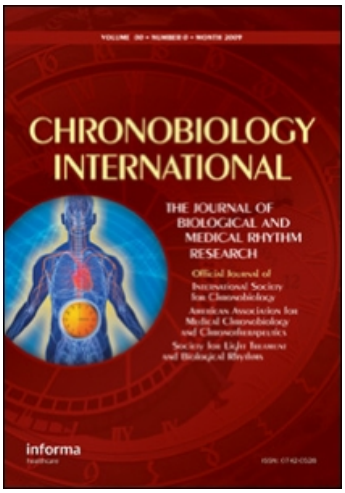

Chronobiology International
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597233

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Online Publication Date: 01 January 2009

To cite this Article Danilenko, Konstantin V., Plisov, Igor L., Wirz-Justice, Anna and Hébert, Marc(2009)'Human Retinal Light Sensitivity and Melatonin Rhythms Following Four Days in Near Darkness',Chronobiology International,26:1,93 — 107
To link to this Article: DOI: 10.1080/07420520802689814
URL: http://dx.doi.org/10.1080/07420520802689814

Chronobiology International, 26(1): 93–107, (2009)

ISSN 0742-0528 print/1525-6073 online
DOI: 10.1080/07420520802689814

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HUMAN RETINAL LIGHT SENSITIVITY AND MELATONIN RHYTHMS FOLLOWING FOUR DAYS IN NEAR DARKNESS

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The rods in the retina are responsible for night vision, whereas the cone system enables day vision. We studied whether rod function in humans exhibits an endogenous circadian rhythm and if changes occur in conditions of prolonged darkness. Seven healthy subjects (mean age ± SD: 25.6 ± 12.3 yr) completed a 4.5-day protocol during which they were kept in complete darkness (days 1 and 4) and near darkness (<0.1 lux red light, days 2 and 3). Electroretinography (ERG) and saliva collections were done at intervals of at least 3 h for 27 h on days 1 and 4. Full-field ERGs were recorded over 10 low-intensity green light flashes known to test predominantly rod function. As a circadian marker, salivary melatonin concentration was measured by radioimmunoassay. The ERG data showed that rod responsiveness to light progressively diminished in darkness (significantly lower a- and b-wave amplitudes, longer b-wave implicit time). The decrease in amplitude (b-wave) from day 1 to day 4 averaged 22 ± 14%. After correction for the darkness-related linear trend, the circadian variations in ERG indices were weak and usually non-significant, with slightly higher responsiveness to light during the day than night. Rod sensitivity (by K index) tended to decrease. Strikingly, the overall amount of melatonin secretion (area under 24 h curve) also decreased from day 1 to day 4 by 33.1 ± 18.9% ($p = .017$). The drift of the melatonin rhythm phase was within the normal range, less than 56 min over three days. There was no significant correlation between the changes in ERG responses and melatonin. In conclusion, scotopic retinal response to (low-intensity) light and the amount of melatonin secreted

Submitted April 23, 2008, Returned for revision July 1, 2008, Accepted September 22, 2008
Supported by grants of the Swiss National Science Foundation Programme "Scientific Cooperation between Eastern Europe and Switzerland" #7SUPJ06228, the Velux Foundation, and the EU 6th Framework Project EUCLOCK (No. 018741).

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are diminished when humans are kept in continuous darkness. Both processes may have a common underlying mechanism implicating a variety of neurochemicals known to be involved in the regulation of both photoreceptor and pineal gland function. (Author correspondence: dani@irs.ru)

Keywords Human rod electroretinography (ERG), Circadian rhythm, Darkness, Melatonin

INTRODUCTION

Studies of the human circadian system, which is known to be very sensitive to photic stimuli, are often performed in continuous dim light, sometimes for as long as two months (Wright et al., 2001). Given that the retina also contains a circadian pacemaker that actively gates photic input to the biological clock in the suprachiasmatic nuclei (Remé et al., 1991; Tosini et al., 2007), it may be retinal sensitivity per se that adapts over the course of a long stay in dim light, potentially influencing the results of these studies. Whether there is a change in retinal sensitivity during the day, and over the course of days in continuous dim light, has not yet been thoroughly established, and may be different for different photoreceptors.

The human retina contains three photoreceptor systems; the classic photoreceptors are rods and the cones, and the novel photoreceptor system is a subset of ganglion cells in the inner retina. Cone photoreceptors are responsible for high visual acuity and color vision, and are subdivided into three categories depending on their peak sensitivity to long, medium, and short wavelengths (558 nm, 530 nm, and 420 nm, respectively). In vivo, these peaks are shifted toward longer wavelengths due to the macula pigment (565 nm, 545 nm, and 440 nm, respectively). Only one type of rod photoreceptor is present in mammals, with a spectral sensitivity peak at 496 nm (green light). In addition to the above classical photoreceptors, a third type of photoreceptor has been recently discovered and corresponds to a subgroup of ganglion cells containing the photopigment melanopsin (peak sensitivity in the blue range ~478–484 nm). These novel photoreceptors have been shown to project to the suprachiasmatic nuclei and other "non-image forming" structures in mammals and are involved in light-induced suppression of melatonin production, circadian phase shifting, and pupillary constriction (reviewed in Hankins et al., 2008; Hannibal, 2006; Revell & Skene, 2007). Experiments in a rodless and coneless subject found that this receptor also—most interesting of all—mediates conscious vision, itself, most likely acting as a rudimentary brightness detector (Zaidi et al., 2007).

When the light level is less than 1 lux (0.034 cd/m^2), vision is restricted to the scotopic domain, and only rods and possibly photoreceptive

ganglion cells contribute to the visual response. Above 10 lux (3.4 cd/m^2), in photopic conditions, visual input is driven by cones and also photoreceptive ganglion cells (Zaidi et al., 2007), as rods are then saturated. Between 1 and 10 lux (an illuminance just sufficient to read), the photoreceptive systems are active to generate so-called mesopic vision.

The best means to objectively characterize retinal sensitivity in humans employs electroretinography (ERG). The ERG allows the recording of bio-potentials originating from the retina in response to brief standardized light flashes. According to international standards (Marmor et al., 2004), in order to assess the photopic system specifically, relatively bright flashes are presented against a white rod saturating background (17–34 cd/m^2), to which the subject is pre-adapted for at least 10 min. To assess the scotopic system, relatively dim light flashes are presented in darkness, to which the subject is pre-adapted for at least 20 min. The function of the novel photoreceptors may also be characterized indirectly, as they appear to regulate the ERG cone-driven responses (Hankins & Lucas, 2002). They cannot be estimated directly, as the ERG in mice with genetically disrupted function of cones and rods is flat (Barnard et al., 2004).

Two studies have addressed the question of whether ERG indices in humans undergo circadian rhythmicity, and both have shown somewhat lower ERG responses during the night and early morning compared to daytime and evening (Nozaki et al., 1983; Tuunainen et al., 2001). These studies used a single flash intensity (which probed in fact mixed rod-cone functions) and were not performed in prolonged darkness. The goal of the present study was to elucidate whether there is an endogenous circadian rhythm of retinal scotopic function as measured under conditions of complete darkness, and whether retinal sensitivity is altered following four days in near darkness. Our hypothesis was that rod sensitivity follows a circadian rhythm, with higher sensitivity during the night, and that sensitivity would be increased by conditions of prolonged darkness. In parallel to the ERG, we measured the circadian rhythm of melatonin (in saliva) to determine internal clock time and its relationship to the ERG.

METHODS

Eight female subjects entered the study, and seven (age between 19–45 yrs; mean ± SD: 25.6 ± 12.3 yrs) completed it. One subject dropped out because of external circumstances. All subjects reported having no physical, psychological, or eye complaints; good visual acuity; normal sleep habits; and to be non-smokers. They were instructed to maintain a regular sleep schedule between 23:00–08:00 h ±1 h for five days prior to the study and to keep a daily sleep log, which was checked upon arrival to the isolation facility. The experimental protocol conformed to

international ethical standards (Portaluppi et al., 2008) and was approved by the Ethics Committee of the Institute of Internal Medicine SB RAMS.

The study was performed between October 2004 and June 2005. The subjects (two per study period) entered the laboratory at 21:00 h on day 0, went to bed at 23:00 h, and remained in complete darkness on days 1 and 4 and in near-darkness on days 2 and 3 over a total of 4.5 days. On days 1 and 4, saliva was collected and rod ERG was performed at intervals of at least 3 h, starting from 09:00 until 12:00 h the next day. During these days, ambient light was 0 lux, except for short periods of time in the bathroom or kitchen (<0.1 lux red LED light) or during the manipulations with electrodes (attachment or checking positioning). The latter manipulations were done using a red LED light <30 lux, provided by a light visor that was directed to the face of a test subject with eyes closed most of the time during these procedures. During measurements, light from the computer monitor was dimmed and shielded completely from the test subjects by an opaque fabric covering the display, head, and hands of the investigator. On days 2 and 3, no measurements were performed, and the ambient red light <0.1 lux was maintained everywhere (i.e., including the two living rooms and kitchen). Sleep was achieved in complete darkness (0 lux) from 23:00–08:00 h but interrupted for ERG assessments on days 1 and 4. The sleep episodes were monitored by wrist actimetry (Gähwiler^{TM}, Zurich).

On the experimental days, the subject's pupils were fully dilated with 1% atropine at 08:15 h or with 1% cyclomed twice a day (08:15 and 22:00 h). Pupil diameter was verified ~40 min after dilation and at the end of the 27 h assessment period. Electrode placement was performed between 08:15–09:00 h. Three gold disc electrodes were placed at the forehead (ground) and the right and left external canthi (references), respectively. Nuprep^{TM} abrasive gel was used to prepare the skin prior to electrode placement, and electrodes were filled with EC2^{TM} electrode cream to improve electrical conductance. The active electrodes were composed of a silver/nylon DTL fiber (Shieldex 33/9 Thread, Statex, Bremen, Germany) positioned deep inside the inferior eyelid of the each eye (as per Hébert et al., 1996). The DTL electrode was well tolerated during the 27 h testing period. The electrode impedance was checked regularly throughout the study and kept at a level <2–3 kOhms.

Each rod ERG assessment lasted for 5 to 8 min. The subject was seated facing a Ganzfeld dome that provided an even stimulation of the entire visual field upon flash presentation. A series of green flashes of increasing intensities were presented (see Table 1). Green was used to better stimulate the rods, as they are most sensitive to light at 496 nm. This color was provided by a Rosco medium blue-green filter (E-color No. 116, peak transmission 500 nm, half bandwidth 438–542 nm) placed in front of the light strobe. The flashes were delivered by a strobe xenon arc flash lamp

TABLE 1 Protocol of rod ERG assessment

Flash intensity, log cd·s/m^2	Flash color	Number of flashes	Flash interval, s	Ganzfeld background, cd/m^2	Number of subjects studied
−4.38	Green	10 or 20	1	0	3
−4.08	Green	10 or 20	1	0	5
−3.78	Green	10	1	0	5
−3.48	Green	10	1	0	7
−3.08	Green	10	1	0	5
−2.76	Green	10	1	0	5
−2.46	Green	10	1	0	7
−2.06	Green	5	2	0	5
−1.76	Green	5	2	0	5
−1.43	Green	5	2	0	7

Shading follows the most studied flash intensities.

clamped on the top of the Ganzfeld dome; the strobe flash was generated by a photic stimulator PS22 (®Grass) that provided flashes at an exact duration (10 μs). Calibrated flash intensities were controlled via neutral density filters and settings on the PS22 stimulator, whereas the interval between flashes and acquisition of the retina electrical response were under the control of the AcqKnowledge 3.7.2 software. The retinal signal was filtered (bandpass set at 1–1000 Hz) and amplified 10,000 times by means of BIOPAC amplifiers (RC Electronic, Inc.). When the recording was compromised by artifacts (due to eye blinking, movements, partially closed eyes, or technical reasons), the flash series was repeated. While the implicit time of the evoked potential does not depend on positioning of the DTL electrode, the amplitude can increase if the electrode moves up from the conjunctival bag toward the cornea. If this was suspected to be the case (e.g., after sleep), the electrode location was checked under red light. Following confirmation, the artifact amplitude from this eye was not taken into account in the calculation; otherwise, it was averaged with the value of the other eye. The characteristics of the rod ERG waveforms were analyzed offline with AcqKnowledge 3.7.2 software. The analysis explored three major components (Brown, 1968): a-wave implicit time (time elapsed from the flash to the trough), b-wave implicit time (time elapsed from the flash to the peak), and b-wave amplitude measured from the trough of the a-wave (or baseline if no a-wave was present) to the peak of the b-wave. The fourth principal component, a-wave amplitude, is not conventionally used in the analysis of rod ERG, as it is unquantifiable from noise at low intensities. At higher intensities, an a-wave can be detected and is usually indicative of a cone contribution to the response. Whereas the a-wave represents photoreceptors (rods and cones), the b-wave originates mostly from retinal neuronal cells

postsynaptic to the photoreceptors. The first measurement at 09:00 h was discarded from the analysis due to procedural artifacts related to the familiarizing of the subjects to the test procedures in darkness.

In order to assess if there were any abrupt changes in ERG indices during the 24 h period, in the first two subjects, the scotopic function was assessed every 1.5 h, but using only three intensities (-1.43, -2.46, and -3.48 log $cd \cdot s/m^2$). When it became clear that there were no abrupt changes, we increased the interval to 3 h between measurements and used a wider range of intensities (see Table 1) in order to generate a luminance response function from which we were able to define the log K parameter. The log K represents the intensity at which the rod system generates half-maximal amplitude response and is interpreted as defining retinal sensitivity. To extrapolate the log K parameter, the b-wave amplitude was plotted against flash intensities and fitted with a sigmoidal function (Origin 7 software, as per Hébert al., 1996) with limits fixed to individual maximal and minimal amplitude values derived from the intensities used in our study.

Approximately 1.5 ml of saliva were collected every 1.5 or 3 h (just before each ERG assessment) and every 0.5 h between 19:30 and 24:00 h on days 1 and 4. The collected saliva samples were centrifuged and kept frozen until radioimmunoassay for melatonin using Bühlmann kits, a method showing high correlation with the serum melatonin assay (Weber et al., 1997). Melatonin from a single study subject was assayed in one run to avoid interassay variability. The coefficient of intra-assay variability was 2–5% (for values within the range of 1–30 pg/ml). A phase marker, dim light melatonin onset (DLMOn $\frac{1}{4}$), was calculated as the time of the $\frac{1}{4}$ amplitude upward crossing during the evening rise after fitting the 24 h melatonin curves with a skewed bimodal baseline cosine function (Van Someren & Nagtegaal, 2007).

Statistical analyses were performed with Statview 4.5 and SuperANOVA 1.11 software for Macintosh. Analysis of variance for repeated measures (rANOVA) was the primary statistics in the study; rANOVA's Huynh-Feldt's corrected probability $p < .05$ was considered as a significant result. Comparison between variables was made with Student's *t*-test and correlation with Pearson's test. Standard deviations (SD) from the means accompany mean values in the text, whereas standard errors of the means (SEM) are presented in the figures.

RESULTS

Rod ERG Following Four Days in Darkness

Figure 1 shows the 24 h variation of the ERG indices in darkness at the three different flash intensities (low, medium, and high) on days 1 and 4. A

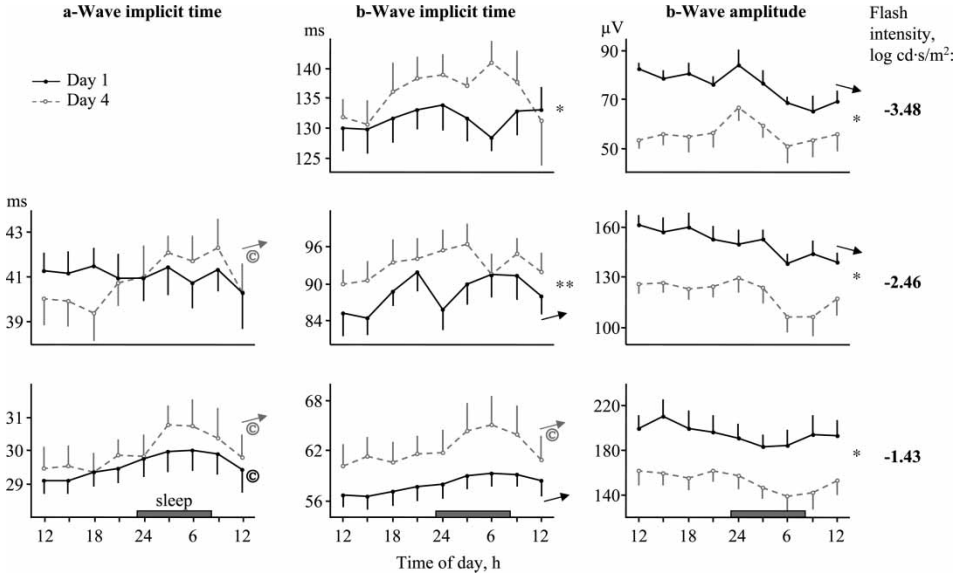

FIGURE 1 Dynamics of rod ERG indices in seven subjects living in (near) darkness for 4.5 days. $^{*}p < .05$, $^{**}p < .01$ indicate a significant difference between mean values on days 1 and 4. ↑ or ↓ indicate significant increasing or decreasing 24 h linear trend (at least $p < .05$). © indicates significant circadian-like variations over the 24 h (after removal of linear trend; one-way rANOVA $p < .05$).

main effect of (near) darkness appeared to be a decrease in the ERG b-wave amplitude. This decline could be already observed during day 1, as reflected by a significant linear trend at some intensities (downward pointing arrows in Figure 1). A linear trend was also observed for the a-wave and b-wave implicit times, which lengthened with the decline in amplitude of the b-wave (upward pointing arrows when significant). On day 4, the ERG response amplitudes were significantly lower than on day 1 (asterisks; averaged decrease of $22 \pm 14\%$, $N = 7$), and the b-wave implicit time was generally increased, whereas the a-wave implicit time was not changed significantly. The day 1 to day 4 findings can readily be seen in the 24 h average waveforms reproduced in Figure 2, from a representative study subject.

To ascertain changes in the a-wave from day 1 to day 4, we analyzed the 24 h averaged waveforms of each subject (as in Figure 2). A significant decrease of the a-wave amplitude was found from 12.6 ± 7.1 to 2.7 ± 2.2 μV ($p = .0058$) and from 40.0 ± 12.7 to 24.7 ± 10.5 μV ($p = .0041$) at intensities of -2.46 and -1.43 log cd · s/m^2, respectively. Again, the a-wave implicit time was not changed significantly.

Visual inspection of the amplitude versus intensity plot (the luminance response function; see Figure 3) suggests that maximal scotopic response was achieved between -2 and -1.5 log cd · s/m^2 on both days, which is

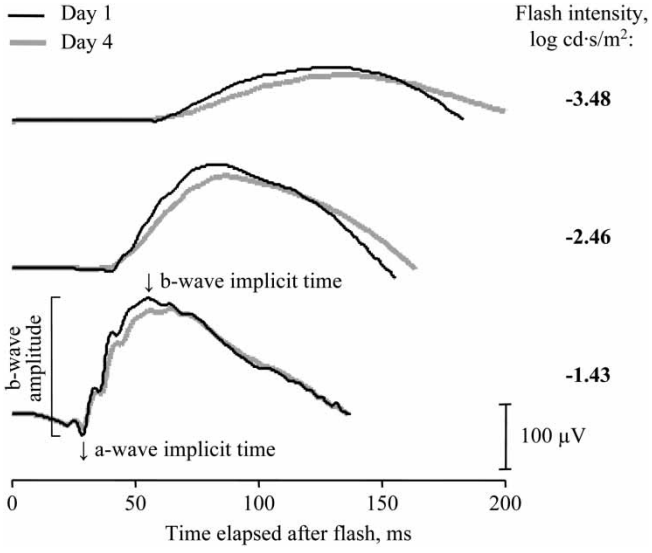

FIGURE 2 Change of ERG waveforms (nine averaged over 24 h) in a representative subject "D" from days 1 to 4 at three different flash intensities.

normal for rod function based on our experience with the spectral flashes used. The log K index—the intensity at which the half-maximal amplitude is attained—tended to increase from day 1 to day 4: -3.14 ± 0.08 vs. -3.09 ± 0.10 log cd · s/m^2 ($p = .090$, n = 5), suggesting a decrease in sensitivity, consistent with the decrease observed in response amplitude.

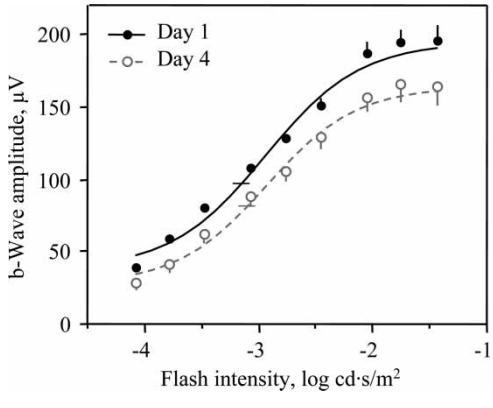

FIGURE 3 The luminance response function after three days in near darkness. The amplitude of ERG responses at each flash intensity was averaged over 24 h and then over five subjects (mean + SEM), plotted against a range of flash intensities, and fitted by a sigmoidal curve. All b-wave amplitudes on day 4 were significantly lower than on day 1 at corresponding flash intensities (at least $p < .05$). — represents the point where the sigmoidal curve crosses half-maximal amplitude response.

Rod ERG Circadian Variations

As seen above, significant rANOVA results could be due to the presence of increasing or decreasing linear trends in darkness, rather than a circadian variation per se. To distinguish the circadian variation from the linear trend, the latter was mathematically removed by correction of each value for the slope coefficient of the individual curves. After correction, the ERG indices exhibited a significant circadian-like variation in 4/16 cases, observed only for the a- or b-wave implicit times, but not amplitude (sign "©" in Figure 1). The a-wave and b-wave implicit times appeared to lengthen at night along with the (non-significant) amplitude decline. The shape of the 24 h variation persisted from day 1 to day 4 (no significant day × time interaction by rANOVA).

Melatonin

Melatonin secretion revealed a classical circadian rhythm with very low levels during the day and a sharp rise between 20:00 and 23:00 h to high nighttime values, with the exception of subject "C" on day 1, whose secretion was elevated both during the day and night (see Figure 4a).

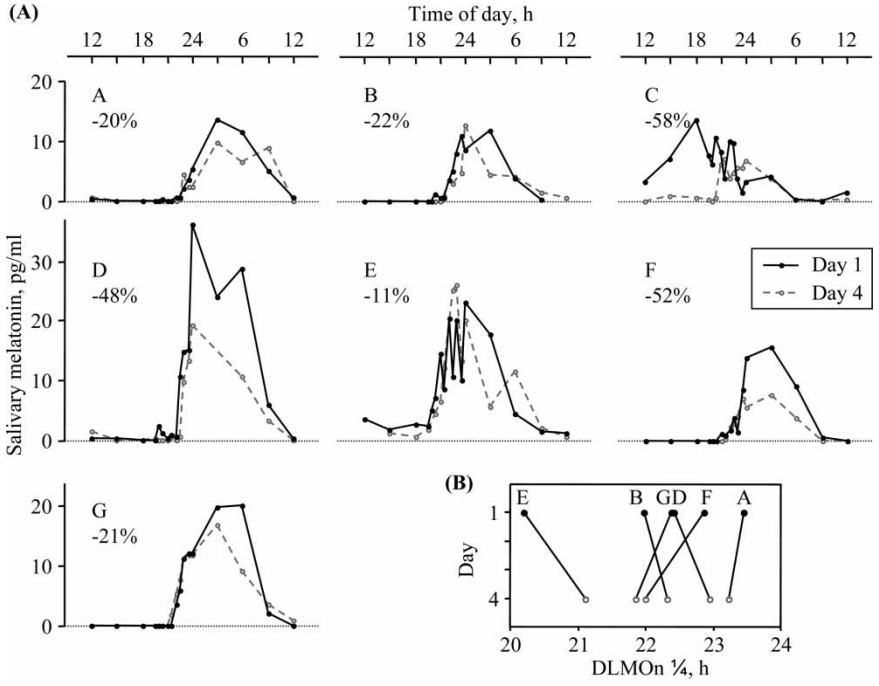

FIGURE 4 (A) Individual profiles of 24 h melatonin secretion and percent decrease of melatonin secreted (area under the curve) from day 1 to day 4. (B) Plot of the shift in DLMOn $\frac{1}{4}$ calculated for each individual (except "C", the DLMOn for which on day 1 was undefinable) following four days in near darkness.

On day 4, the overall amount of melatonin secreted was diminished; the area under the curve decreased significantly from day 1 to day 4 ($p = .017$) by $33.1 \pm 18.9\%$, and this reduction occurred in all seven study participants. After three days in near darkness, the expected drift in phase (using DLMOn $\frac{1}{4}$ as phase marker) was apparent, ranging from a small advance to small delay, of magnitude less than 56 min over the entire period. The only exception was of subject "C," for which DLMOn on day 1 was undefinable (see Figure 4b). There was no significant correlation between the change (%) in the total amount of salivary melatonin over the 24 h and the change in the ERG indices ($p > .52$).

DISCUSSION

Our human study resulted in three main findings, two of which were unexpected, striking, and unequivocal: the rod responsiveness to light and the 24 h salivary melatonin concentration were both decreased following four days in near darkness (<0.1 lux red light). The third finding was that there is only a weak, if any, endogenous circadian rhythm in rod responsiveness to light.

Rod ERG Following Four Days in Darkness

We expected an increase of rod sensitivity response, as it would have paralleled the subjective increase of the rod-driven light sensitivity observed with dark adaptometry in people maintained in complete darkness for many days using an eye patch (Clark et al., 1946). We were even more surprised that rod function appeared to decrease. In the only other human ERG study in which subjects were maintained in constant darkness for 14 h beginning at midnight (n = 3), there was a progressive shortening, not prolongation, of b-wave implicit time, and no change in amplitude (Hankins et al., 2001). However, when we carefully compare our findings, the discrepancy can be resolved. The flashes applied in the Hankins et al. (2001) study were much longer and brighter, thus testing the cone rather than the rod system. When looking at the brightest flash intensity in our study (-1.43 log cd $\cdot$ s/m^2), there was also a shortening of the b-wave implict time from night to day (circadian-dependent) without significant change in amplitude (see Figure 1). In animals, however, there are numerous studies showing lower ERG response in DD (dark:dark) cycles compared to LD (light:dark) cycles (e.g., Hamasaki & Pollack, 1972). A close inspection of the studies in which ERG dynamics in DD were also studied brings some support for our data. Though not accentuated in their article, Figures 6c and 8a in Miranda-Anaya et al. (2002) showed a decreased ERG response following three days in darkness in the

day-active iguana. A similar effect was described in Japanese quail following two days of complete darkness (Manglapus et al., 1998).

The mechanism of decreased ERG response in continuous darkness is unclear. The a-wave represents photoreceptors and the b-wave next-order retinal neuronal cells. Both waves were diminished, suggesting a primary role of photoreceptors. One possibility is that keeping subjects in near darkness does not allow the light-triggering effect of rod disk shedding that normally peaks at dawn (Nguyen-Legros & Hicks, 2000). Older disks that are not phagocytosed may not function normally, leading to a decrease and slowing of the rod response to light.

More than 10 neurochemicals regulate rod photoreceptor function within the retina. Dopamine is probably the most studied (Witkovsky, 2004). Rods have receptors (D2-like) to dopamine for which persistent synthesis and release by retinal amacrine cells are stimulated by light. Findings on the dopamine effects on rod ERG are few and inconsistent (for review, see Witkovsky, 2004). In humans, dopamine antagonists may suppress both a- and b-waves and prolong their implicit times (Bartel et al., 1990) or reduce b-wave amplitude only (Holopigian et al., 1994). Though there is no solid evidence that the overall amount of retinal dopamine is affected following several days in constant darkness (Jaffe et al., 1991; Manglapus et al., 1999), this does not mean that its turnover is not decreased, as shown by the decrease of its metabolites after transition from LL to DD (Doyle et al., 2002).

Studies on other retinal neurotransmitters and neuromodulators also report changes in constant darkness. GABA tonic release, suppressing rod function, is greater in darkness (Boatright et al., 1994); norepinephrine disappears in darkness (Jaffe et al., 1991), and the number of VIP+ cells declines linearly in darkness (Herbst & Thier, 1996). However, as far as we know, none of these neurochemicals has been studied with regard to the rod ERG. The issue may even be more complicated, as the impact on rod functioning might be due to an interaction between several neurochemicals.

Melatonin

There has been no previous report that melatonin secretion changes in humans maintained in dim-light laboratory conditions. In a study by Gronfier et al. (2004), where the entire 24 h melatonin profile was measured under the lowest level of light used so far (<1.5 lux), there was no statistically significant change of melatonin released (area under the 75% upper part of the curve) over three days ($n = 7$; C. Gronfier, personal communication). However, this was still not complete darkness, and not red (but white) light. It is interesting to note that the melatonin amplitude increases with increasing daytime light intensity (Mishima et al., 2001), the opposite of our finding of a decrease in near-darkness.

Animal studies have shown that, depending on species, pineal melatonin production in continuous darkness (DD) can decline, remain unchanged, or even increase. For instance, in rhesus monkeys, the melatonin rhythm persisted during 6.5 days in DD (Reppert et al., 1981). The decrease in pineal melatonin is unlikely to be the cause of the decrease in rod ERG. Exogenous melatonin does not have any impact on rod, low-flash ERG in dogs (Rosolen et al., 2004), though it would be more relevant to test rod ERG after inhibition of melatonin secretion (e.g., by propranolol). The absence of a significant correlation between the decrease in rod ERG and pineal melatonin production in near darkness additionally suggests that these two processes are unrelated. Nevertheless, both may have a common underlying mechanism, as they are regulated by common neurotransmitters and neuromodulators, such as glutamate, GABA, norepinephrine, serotonin, VIP, vasopressin, and neuropeptide Y.

Rod ERG Circadian Variations

The endogenous 24 h fluctuation in rod ERG was not as robust as that observed for the melatonin rhythm, so that no individual circadian phase marker could be derived. The pattern of these very weak variations—characterized by a lower response during night-morning and higher response during daytime-evening—is in accordance with previous findings. Nozaki et al. (1983) recorded rod ERG in 14 healthy subjects at 6 h intervals over a 24 h period and found lowest b-wave amplitude at 06:00 h and highest amplitude at 12:00 h. Data on implicit times were not reported. Using ultrashort 90 min dark [sleep]:light [wake] cycles for 36 h, Tuunainen et al. (2001) found in 12 healthy volunteers a significant circadian rhythm in b-wave implicit time only (longer in the early morning). This design of evenly distributed sleep episodes across 36 h suggests that the circadian variations in ERG we found in our study were not due to the influence of night-time sleep. In six healthy subjects monocularly patched from 20:00 to 08:00 h for seven days, Birch et al. (1984) found a minimum rod ERG response in the entrained but not the unentrained eye at 09:45 h compared to 07:45 h, 16:00 h, and/or 19:00 h. This latter finding was interpreted to result from the disk shedding process occurring 1.5 h after light onset. However, the design of that study was not ideal to measure circadian rhythmicity.

Because circadian variations in rod ERG indices are very weak and appeared mainly at brighter flash intensities, it is possible that they are due to cone input to the rod response. A small number of cones contribute to the rod ERG based on analysis of the a-wave in humans (Hood & Birch, 1994). At low flash intensities, when the a-wave is undetectable, the extent of cone contribution seems to be negligible (as follows from Figure 3 in Michaelides et al., 2004). There is, in fact, some evidence from the

animal literature that endogenous circadian variations in the ERG are due to cones and not rods (Manglapus et al., 1998). Cone function fluctuations, in turn, are influenced by melanopsin-containing photoreceptive retinal ganglion cells, as in melanopsin knockout mice, as the normal day-night difference in the cone ERG in melanopsin knockout mice is abolished (Barnard et al., 2006).

In conclusion, the obtained findings—a decrease in scotopic retinal function (ERG) and a decrease in overall melatonin following four days in (near) darkness—are novel for humans. Indeed, continuous darkness may be an extraordinary, non-physiological condition as compared with the scotopic condition of <1 lux white light used to test retinal and pineal function. More studies are needed to better understand how the lack of light may impact retinal and also circadian functioning.

ACKNOWLEDGMENTS

The authors thank Mark Hankins and Rob Lucas for proposing the protocol of this study and for preliminary discussion of the methods and results. We are grateful to the test subjects for their participation in the study, Elena Danilenko and Sergei Kondratov for their help in running the study, Jakob Weber and Ekaterina Semenova for melatonin assays, Eus Van Someren for calculations of DLMOn $\frac{1}{4}$, and anonymous referees for their helpful suggestions.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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