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Human Cone Light Sensitivity and Melatonin Rhythms Following 24-hour Continuous Illumination

Konstantin V. Danilenko,[1] Igor L. Plisov,[2] Howard M. Cooper,[3,4] Anna Wirz-Justice,[5] and Marc Hébert[6]

[1]Institute of Internal Medicine, Siberian Branch of the Russian Academy of Medical Sciences, Novosibirsk, Russia, [2]The Academician S.N. Fyodorov Federal State Institution "Intersectoral Research and Technology Complex 'Eye microsurgery' of Rosmedtechnology," Novosibirsk, Russia, [3]Department of Chronobiology, Stem Cell and Brain Research Institute, INSERM, Bron, France, [4]University of Lyon, Lyon, France, [5]Centre for Chronobiology, Psychiatric Clinics, University of Basel, Switzerland, [6]Faculty of Medicine, Centre de Recherche Université Laval Robert-Giffard, Quebec, Canada

This study investigates the possibility of an endogenous circadian rhythm in retinal cone function in humans. A full-field cone electroretinogram (ERG) was performed every 2 h for 24 h under continuous rod-saturating ambient white light (53 ± 30 lux; pupils dilated) in nine healthy subjects. Distinct circadian variations were superimposed upon a gradual decrease in cone responsiveness to light, demonstrated most reliably in the implicit times of b-wave and oscillatory potentials, and to a lesser extent in amplitude and a-wave implicit times. After mathematical correction of the linear trend, the cone response was found to be greatest around 20:00 h and least around 06:00 h. The phase of the ERG circadian rhythm was not synchronized with the phase of the salivary melatonin rhythm measured the previous evening. Melatonin levels measured under constant light on the day of ERG assessments were suppressed by 53% on average compared to melatonin profiles obtained previously under near-total darkness in seven participants. The progressive decline in cone responsiveness to light over the 24 h may reflect an adaptation of the cone-driven retinal system to constant light, although the mechanism is unclear. The endogenous rhythm of cone responsiveness to light may be used as an additional index of central or retinal circadian clock time. (Author correspondence: kvdani@mail.ru)

Keywords: Circadian rhythm, 24-h constant light, Human cone electroretinography (ERG), Melatonin

INTRODUCTION

Circadian variations in retinal sensitivity as measured with the electroretinogram (ERG) have been demonstrated in both invertebrate and vertebrate species, for example, *Limulus* and crayfish (Barlow, 1983; Solís-Chagoyán et al., 2008; Verde et al., 2007), fish (Dearry & Barlow, 1987; Ren & Li, 2004), lizards (Miranda-Anaya et al., 2002), birds (Manglapus et al., 1998; Peters & Cassone, 2005; Wu et al., 2000), mice (Cameron et al., 2008a), rabbits (Brandenburg et al., 1983; White & Hock, 1992), and humans (Danilenko et al., 2009; Tuunainen et al., 2001). In human studies, in which a mixed rod and cone ERG was assessed at several times over the entire 24-h period, retinal responses to light have been reported to increase in the evening and decrease in the morning (Nozaki et al., 1983; Tuunainen et al., 2001). For the rod system, specifically, we reported small-amplitude circadian variation in responsiveness to light in the human eye following 4 days in darkness (Danilenko et al., 2009). For the cone system, another group found no circadian variation in subjects maintained in continuous light (~15 lux) for 24 h, albeit the sample size was very small (n = 3; Hankins et al., 1998).

In the present study we specifically targeted the cone responses to light by monitoring the ERG response during a 24-h period, while subjects were maintained under a constant lighting condition to avoid a "masking" influence of ambient light changes on a possible endogenous circadian rhythm in photoreceptor sensitivity. Parallel to the ERG assessment, the 24-h melatonin variation was assessed to determine internal clock time and its relationship to possible cone ERG circadian variations. During continuous light exposure, the subjects' pupils were dilated and the ambient light intensity was set to ~53 lux at eye level. This intensity corresponds to the minimal light intensity recommended by International Society for Clinical Electrophysiology of Vision (ISCEV) standards to isolate cone from rod ERG responses (17–34 cd/m^2 or ~53–106 lux; Marmor et al., 2009). In addition, 53 lux light was considered sufficiently

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Address correspondence to Dr. Konstantin V. Danilenko, MD, Institute of Internal Medicine SB RAMS, Bogatkova 175/1, Novosibirsk 630089, Russia. Tel./Fax: (007)-383-2642516; E-mail: kvdani@mail.ru

low to avoid complete suppression of melatonin according to results obtained in subjects with fully dilated pupils, in which a 60% melatonin suppression was observed at ~50 lux (Brainard et al., 1988). Maintenance of the expression of melatonin secretion was important in order to allow assessment of its endogenous circadian rhythm.

METHODS

Nine healthy subjects entered and completed the study (1 male, 8 female; age between 20 and 49 yrs; mean ± standard deviation: 25.6 ± 9.1 yrs). Seven had participated in previous studies: six in a "Rod ERG in near-darkness" study (Danilenko et al., 2009) and one in a "Sleep-phase advance" study (Danilenko et al., 2003). No subjects reported physical or psychological complaints, ocular problems, acute illness, or transmeridian travel during the preceding month, and all had normal sleep habits. They were instructed to maintain a regular sleep schedule between 23:00 and 08:00 h ± 1 h for the 5 days prior to the study and to keep a daily sleep log, which was checked upon arrival in the isolation unit. The experimental protocol conformed to international ethical standards (Portaluppi et al., 2010) and was approved by the Ethic Committee of the Institute of Internal Medicine of the Siberian Branch of the Russian Academy of Medical Sciences (SB RAMS).

The study was performed in October 2008. The subjects completed a 39-h protocol (in groups of three). They entered the isolation unit at 19:00 h (Day 1), slept in darkness from 23:00–08:00 h, and stayed continuously awake (Day 2) from 08:00 h until 10:00 h the next day under ambient light (53 ± 30 lux). Broadband-white light was provided by a combination of light fixtures equipped with fluorescent and tungsten lamps on the ceiling of each room. The wakefulness of the subjects was maintained by sedentary games, radio, TV, computer, conversations, and continuous interactions and observation from the personnel.

Full-field flash ERG was performed every 2 h starting at 10:00 h for 24 h as described elsewhere (Danilenko et al., 2009). Briefly, the subject's pupils were dilated with 1% Cyclomed during placement of skin electrodes on the forehead (ground) and each canthus (references) between 08:15 and 09:00 h. Drops were reapplied in the evening to maintain dilatation and the pupil size was measured 4–6 times over 24 h to ensure consistency. A metallic thread proposed to be used as eye electrode by Dawson, Trick & Litzkow (1979) is hence named "DTL." A DTL fiber (Statex, Bremen, Germany) was positioned deeply in the conjunctival bag of both eyes (as per Hébert et al., 1996) and served as the active electrode. As shown previously, this electrode positioning is well tolerated even when worn for a very prolonged period of time (Danilenko et al., 2009). ERG was recorded using a photic stimulator PS22 (Grass) and BIOPAC amplifiers (RC Electronic) while the subject sat facing a Ganzfeld dome that allowed a complete uniform stimulation of both eyes. Series of short (10 μs) white flashes of defined intensity (Figure 1A) and number (20, 10, or 5 with interflash interval of 1 s) were presented against the Ganzfeld 53-lux (17-cd/m^2) background to which the subject was preadapted for 3 min. Each ERG assessment lasted for 3–4 min. The recorded light-evoked potentials were filtered with a bandpass of 1–1000 Hz and presented graphically (average tracing) with AcqKnowledge 3.7.3 software. Each waveform was further filtered off-line with a low-pass 75 Hz cutoff filter to remove oscillatory potentials in order to derive the major components of the ERG trace, free of the confounding effect of the oscillatory potentials (Figure 1B). These major components were amplitude and implicit time of the a-wave (negative deflection, reflecting photoreceptors) and amplitude and implicit time of the b-wave (positive, large deflection reflecting retinal cells postsynaptic to the photoreceptors). Extracted (bandpass 75–300 Hz) oscillatory potentials were also analyzed at flash intensity 1.20 log cd·s/m^2 as they are generated by different cellular components of the retina, most likely the amacrine cells interneurones (Wachtmeister, 1998). The ERG amplitude might increase due to displacement of the DTL fiber (due to eye blinks, movements); therefore, the ERG implicit time was primarily explored in our analysis as this index is not influenced by DTL electrode positioning. Reliable data from left and right eyes were averaged.

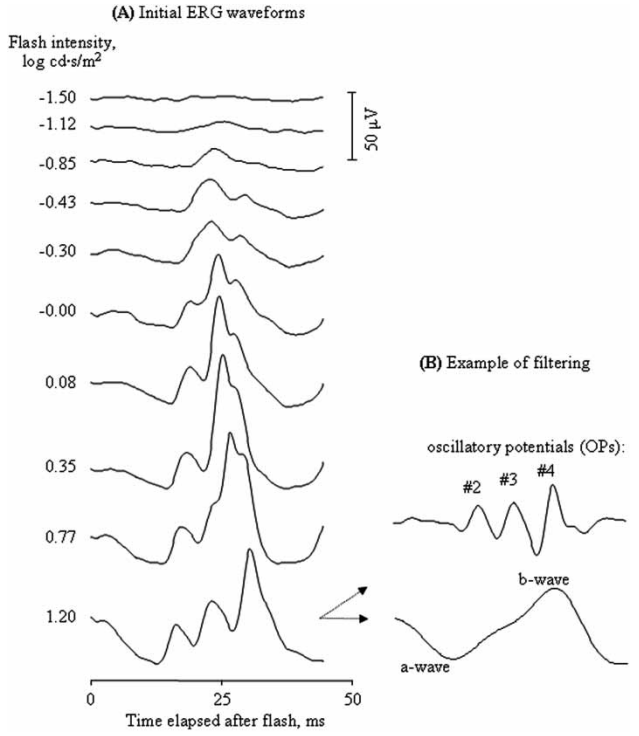

FIGURE 1. (A) Representative waveforms of averaged electroretinographic responses to flashes of different intensities (subject "f"; ERG assessment at 18:00 h). (B) Each waveform was filtered out from the oscillatory potentials for the analysis; the oscillatory potentials were analyzed separately (at intensity 1.20 log cd·s/m^2).

Saliva for melatonin assay was sampled on Day 1 every hour from 19:00 to 23:00 h, and on Day 2 every 2 h between 10:00 and 18:00 h, every 30 min between 19:30 and 23:00 h, and every 1 h between 00:00 and 10:00 h. The samples (~1.5 mL) were centrifuged and kept frozen until radioimmunoassay of melatonin using Bühlmann kits (Weber et al., 1997). Melatonin from a single 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). The rise of melatonin secretion was determined on Day 1 evening curves by interpolating the time of the upward crossing above the 3 pg/mL threshold, and this served as a circadian phase marker.

Statistics included analysis of variance for repeated measures (rANOVA; SuperANOVA 1.11 software for Macintosh), area under curve calculation (GraphPad Prizm 3.0 software), 3-parameters 24-h sine fit (SigmaPlot 9.0 software), and regression/comparison analysis (Statview 4.5 software). rANOVA Huynh-Feldt corrected probability $p < .05$ was considered significant.

RESULTS

Figure 2A shows the 24-h dynamics of the b-wave implicit time at flash intensity 0.35 log cd·s/m^2 (yielding subthreshold ERG response; Rufiange et al., 2002). The dynamics manifested a significant linear component increase ($p < .01$, Student's t-test; Figure 2B) when connecting the lines between the first and the last points of the individual curves, i.e., the cone response to light progressively taking more time to reach its peak over the 24-h recording. After removal of this linear trend by adjusting the last recording value at 10:00 h to equal the first recording value obtained 24 h earlier and by correcting the values in between with the individual slope coefficient, a significant circadian variation was revealed (Figure 2C). The b-wave implicit time was shorter in the afternoon-evening (minimum at 20:00 h) and longer between the night and early morning (maximum at 06:00 h).

The linear and circadian components for the b-wave implicit time dynamics were significant for most flash intensities tested (Table 1). For the b-wave amplitude (after excluding one subject due to unreliable amplitude recordings resulting from large variation in the DTL position), some significant results also emerged, confirming the progressive decrease in cone responsiveness to light over the 24 h (linear) and the lower responsiveness at night versus day (circadian). At an intensity of 0.77 log cd·s/m^2, which appeared to trigger the maximal b-wave amplitude of about 100 μV in averaged subjects (based on the unfiltered ERG waveforms), the magnitude of the circadian variation was roughly 10 μV, equivalent to roughly a 10% difference. Analysis of the a-wave implicit time yielded few significant results (Table 1), whereas none could be observed with the a-wave amplitude, probably due to the smaller size of the a-wave compared to the b-wave, making changes more difficult to quantify in our small sample.

The results described above were generally similar when ERG responses were not filtered from the oscillatory potentials (OPs). Oscillatory potentials, however, followed the patterns of the ERG a- and b-waves, demonstrating linear and circadian components in implicit times and amplitudes over the 24-h period, the

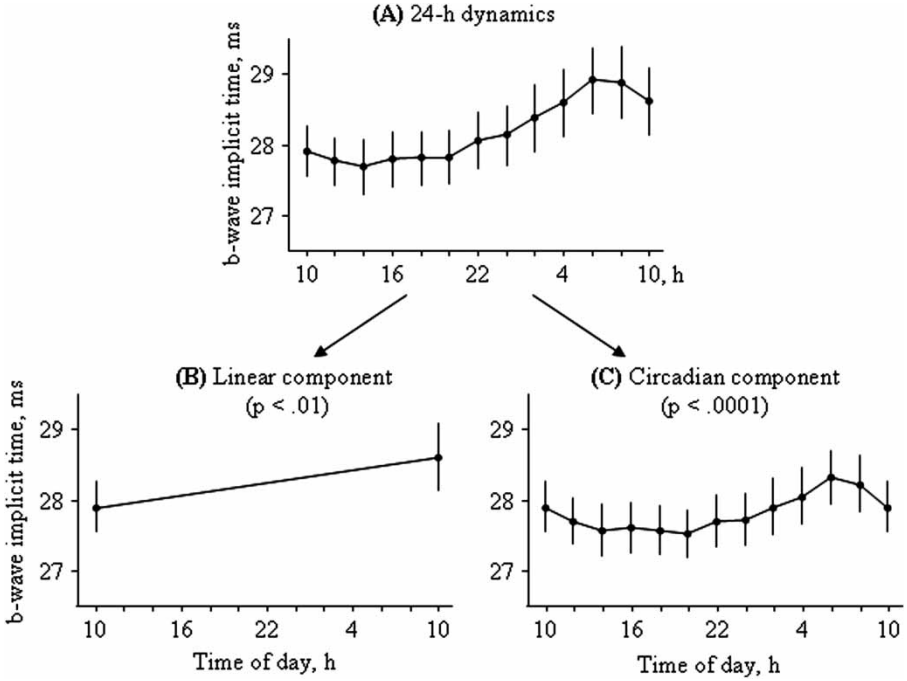

FIGURE 2. (A) 24-h dynamics of b-wave implicit time at ERG flash intensity 0.35 log cd·s/m^2 in nine subjects (mean ± standard error). The dynamics were broken down into (B) linear and (C) circadian components. Both were significant.

TABLE 1. Significance of the dynamics of the major cone ERG indices following 24-h continuous light in healthy subjects (n = 9)

	a-Wave implicit time		b-Wave implicit time		b-Wave amplitude (n = 8)	
Flash intensity, log cd·s/m^2	Linear trend (increasing)	Circadian component	Linear trend (increasing)	Circadian component	Linear trend (decreasing)	Circadian component
–1.50	N/A	N/A	N/A	N/A	N/A	N/A
–1.12	N/A	N/A	N/A	N/A	N/A	N/A
–0.85	N/A	N/A	N/A	N/A	N/A	N/A
–0.43	**0.0016**	0.78	**0.049**	0.81	0.91	0.15
–0.30	0.13	**0.037**	0.099	**0.041**	0.14	0.069
–0.00	0.25	0.37	0.18	**0.0076**	**0.038**	0.35
0.08	0.058	0.90	**0.018**	**0.0001**	0.064	0.066
0.35	0.41	0.13	**0.0058**	**<0.0001**	**0.0085**	0.086
0.77	0.22	0.40	**0.0011**	**<0.0001**	**0.0015**	**0.0073**
1.20	0.072	0.055	**0.027**	**<0.0001**	0.094	**0.038**

N/A = not applicable since responses were not quantifiable at low flash intensities. Values in bold represent significant *p* values calculated for the linear trend by Student's *t* test (comparison of the two 24-h-apart extreme values) and for the circadian component by rANOVA.

TABLE 2. Significance of the dynamics of cone ERG oscillatory potentials (OPs; at flash intensity 1.20 log cd·s/m^2) following 24-h continuous light in healthy subjects (n = 9)

	Time of the negative peak		Time of the positive peak		Amplitude (n = 8)	
	Linear trend (increasing)	Circadian component	Linear trend (increasing)	Circadian component	Linear trend (decreasing)	Circadian component
OP 2	0.68	**0.021**	0.92	**0.016**	0.37	0.44
OP 3	**0.022**	**0.0006**	**0.0016**	**<0.0001**	0.21	0.10
OP 4	**0.0050**	**<0.0001**	**0.0027**	**<0.0001**	**0.0025**	**0.019**

Values in bold represent significant *p* values calculated for the linear trend by Student's *t* test (comparison of the two 24-h-apart extreme values) and for the circadian component by rANOVA.

prominence of which increased with the increase in the sharpness of the oscillatory potentials (from OP 2 to OP 4; Figure 1B; Table 2). There was a highly significant correlation between the 24-h dynamics in the parameters of the OPs and corresponding a- and b-wave parameters.

The observed variations in the ERG could not be correlated with the circadian rhythm of melatonin secretion, since the secretion of melatonin was suppressed by the 24-h continuous ambient light (53 ± 30 lux; pupils dilated). Relative to previous measurements in darkness (Day 1 from the study by Danilenko et al., 2009; n = 6) or near-darkness (<0.2 lux at Day 9 in the study by Danilenko et al., 2003; subject "c"), the average decrease of the 24-h area under the curve was 53% ($p < .05$, n = 7; Figure 3A). In addition, there was considerable intersubject variability, ranging from almost 100% suppression to even an increase of melatonin levels (in subject "h"; Figure 3A). Unfortunately, sleepiness was not measured in the study, but short sleep episodes were indeed documented by research staff in subjects "h" and "a" between 03:00 and 05:00 h. These two subjects had the least changes in melatonin secretion. On average, melatonin suppression was less pronounced at the end of the night between 04:00 and 08:00 h (Figure 3B). This may be related to the occurrences of microsleep (closed eyes), since signs of sleepiness (eyes rolling, complaints of tiredness, etc.) were common at that time, or a diminished effect of light on melatonin secretion.

An attempt was also made to relate circadian phase in ERG (Day 2) to melatonin phase on the previous Day 1 (pupils yet nondilated), as light intensity of ~53 lux on Day 1 was well below the average threshold for melatonin suppression with nondilated pupils, found with 100 lux (Gaddy et al., 1993) or 80 lux (Zeitzer et al., 2000). In four of the nine subjects, melatonin secretion during Day 1 did not rise in the evening during the presleep sampling episode that ended at 23:00 h, rendering impossible determination of the melatonin circadian phase in these participants. When comparing the subgroups of subjects with the melatonin rise starting before 23:00 h versus after 23:00 h, no significant difference was found with respect to the ERG circadian phase. The best result was obtained when the ERG-phase assessment was based on the averaged circadian dynamics of the implicit time of the negative and positive peaks of oscillatory potentials 2, 3, and 4 ($p = .086$, Mann-Whitney test; Figure 4). The circadian phase was determined using a 24-h sine approximation of the circadian curves. According to above averaged circadian rhythms of the OPs, the phase varied between 21:08 and 01:43 h (50% up-crossing time of the fitted sine curves). The difference between the ERG circadian

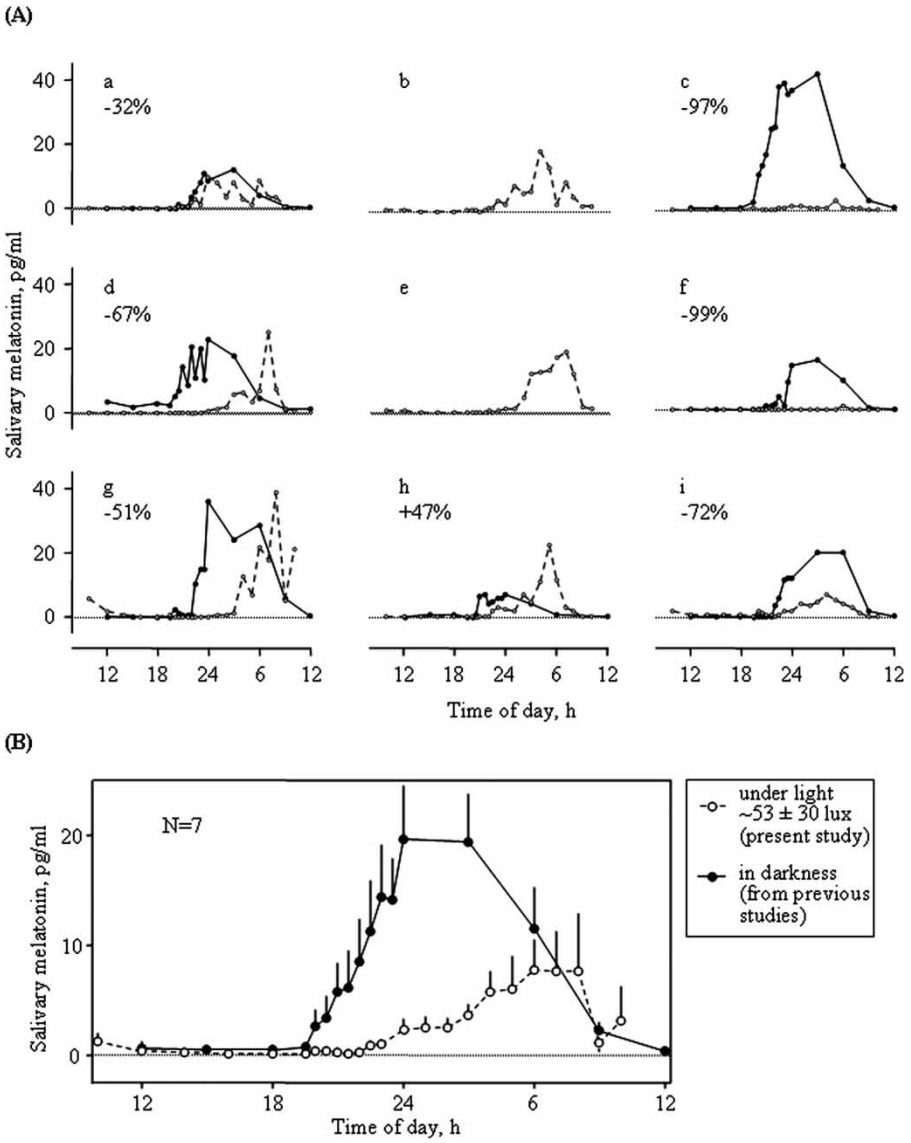

FIGURE 3. (A) Individual profiles of 24-h melatonin secretion under dim light of 53 ± 30 lux (pupils dilated) compared to profiles recorded in darkness. Percent difference (area under the curve) is indicated. (B) Average melatonin profiles (mean and standard error) in seven subjects for whom data from both continuous darkness and continuous light were available.

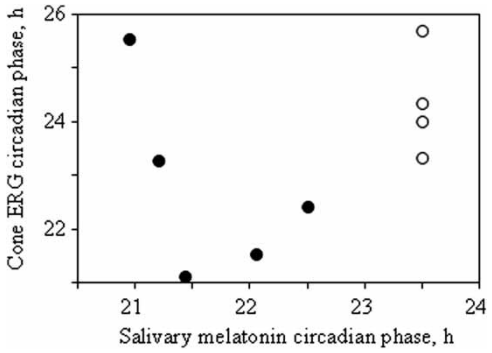

FIGURE 4. Relationship between the circadian phases measured with melatonin and ERG. Open circles represent a subgroup of subjects in which melatonin secretion had not yet begun at 23:00 h and, therefore, the phase being later, could not be determined and was arbitrarily assigned to 23:30 h. The two subgroups did not differ significantly with respect to the ERG circadian phase (see text for details).

phase and melatonin circadian phase ("phase angle") ranged from –0.51 to 4.61 h (n = 5).

DISCUSSION

This study revealed a progressive decrease in cone responsiveness to light following 24-h constant light together with distinct circadian variations: higher responsiveness was recorded in the early evening and decreased responsiveness was recorded in the early morning. These findings were most significant for the ERG b-wave and oscillatory potential implicit times.

A reduction in the cone ERG over a 30-h exposure to constant light has been reported previously in mice (Cameron et al., 2008a). The underlying mechanism of the cumulating effect of increasingly long prior illumination is, however, unclear. In our previous human ERG

study, we also found a progressive decrease in rod responsiveness to light following 4 days in near-total darkness (Danilenko et al., 2009), an effect that has also been described in the day-active iguana (Miranda-Anaya et al., 2002) and Japanese quail (Manglapus et al., 1998). The decrease in the responsiveness to light under constant light conditions may thus have a similar underlying mechanism for the rod and cone pathways.

Our study comprehensively describes an endogeneous circadian rhythm in the cone-mediated retinal light responses in humans. These temporal variations were certainly not due to changes in pupil size following topical application of the mydriatic, since the drops were applied both at ~8:30 and ~22:00 h, whereas the ERG changes went in the opposite directions (increased response from 10:00 h and decreased response from 22:00 h). The pattern of these variations was similar to that of rods assessed in darkness (Danilenko et al., 2009), but the magnitude was much more pronounced. Two previous studies that exploited mixed cone and rod ERGs assessed at several times over the 24-h period showed a similar increase in the evening versus a decrease in the morning of the retinal response to light (Nozaki et al., 1983; Tuunainen et al., 2001). This evening-morning difference is also corroborated by mouse studies that assessed cone ERGs two to four times during a period of 24-h constant light (Barnard et al., 2006; Cameron & Lucas, 2009; Cameron et al., 2008a; Storch et al., 2007). In one human study, however, no circadian ERG rhythm was found in three subjects maintained under ~15 lux for 24 h (Hankins et al., 1998). This could be due to the small sample size and/or the fact that very bright red-light flashes were used, which differ from the white flashes used in our investigation.

The findings in the present study were consistently significant for the ERG b-wave and OP waves implicit times but not for amplitudes. The discrepancies could be explained first by technical issues. As mentioned in Methods, implicit time does not depend on the position of the active electrode in the eye, and thus it was the most reliable ERG study parameter. The b-wave amplitude usually changes in tandem with b-wave implicit time (the opposite relationship), but that was not the case in some of our nine subjects, most likely due to some shifting in the DTL position over the 24-h recording. Evidence for this is the fact that the amplitude sometimes differed between the two eyes in the same subject (while pupil size remained unchanged), suggesting the DTL fiber in one of the eyes moved slightly during the 24-h period it was worn. The a-wave shape depends on the overlapping b-wave: "a reduction in b-wave implicit time would result in an earlier truncation of the a-wave," and this could, in theory, compromise amplitude and implicit-time expression of this smaller wave on the ERG trace (Cameron et al., 2008b). Due to these technical limitations, the results on amplitude of the ERG waves and a-wave implicit time, albeit significant, need to be interpreted with caution.

The distinctness of circadian variations in the implicit times allowed us to calculate the individual circadian phase of the rhythm in each of the nine subjects. Using calculations based on OP 2, 3, and 4 implicit times, the phase covered a range of roughly 4.5 h that matched the normal interindividual range in internal phase measured with melatonin secretion. The ERG phase (early versus late) did not match well with the melatonin phase (early versus late) of our subjects. This may be due to the small sample size of the study, technical reasons (insufficient sensitivity of the ERG method and reliability of phase assessment with the sine function), typical between-subject variabilities in phase difference (i.e., circadian phase angle), and/or atypical melatonin secretion on the day of the ERG assessments due to its suppression by light. Although the magnitude of the melatonin suppression (53%) by the ambient light during the night was very close to the expected 60% decrease based on the Brainard et al. (1988) study, the suppression showed large interindividual differences. In accordance with our results, it has been recently shown that overnight white light of 150–600 lux (pupils not dilated) suppressed melatonin by 53% when compared to dim light (<5 lux), also with large interindividual differences (Van de Werken et al., 2009).

The circadian variations in cone responsiveness to light in humans are obvious, and further studies are needed to investigate how the rhythm is regulated by the central (hypothalamic) and retinal cells clocks (Ruan et al., 2008). The retinal clock network regulates diverse aspects of retinal physiology, including gene expression, rod-cone coupling, disc shedding, dopamine and melatonin release (Doyle et al., 2002; Grace et al., 1996; Remé et al., 1991; Ribelayga et al., 2008; Tosini et al., 2008), all of which are interrelated and can potentially affect the cone ERG. An endogenous mechanism appears to be involved in the 24-h variation of ERG responses, since it can be phase shifted by melatonin in several species (Peters & Cassone, 2005; Solís-Chagoyán et al., 2008) and reduced or lost in mice with invalidation of essential clock genes *Bmal1* or *Cry1*/*Cry2* (Cameron et al., 2008a; Storch et al., 2007) or with deficiency of MT1 melatonin receptors (Baba et al., 2009). The circadian rhythms of retinal dopamine and melatonin release are affected by ambient light and express a circadian rhythm in constant light conditions (Miranda-Anaya et al., 2002; Wirz-Justice et al., 1984). These two neurochemicals are mutually inhibitory and in antiphase, with dopamine being produced during the day (or subjective day) and melatonin at night (or subjective night; Tosini et al., 2008). Increased dopamine levels during the day favors a decreased coupling between AII amacrine cells and cone ON-bipolar cells, whereas melatonin can affect photoreceptor membrane conductance (Cosci et al., 1997). Dopamine also exerts strong influences on rod-cone electrical coupling that is increased at night but blocked during the day (Ribelayga et al., 2008). Dopamine and melatonin affect the amplitude of the

ERG b-wave in an antagonistic and phase-dependent manner in lizards (Miranda-Anaya et al., 2002). Exogenous melatonin prolongs a- and b-wave implicit times and decreases a- and b-wave amplitudes in day-active species, for example, in the chicken (Peters & Cassone, 2005), and in humans it also causes a decline in the ERG response (Emser et al., 1993; Gagné et al., 2009).

Finally, the photopigment melanopsin, which is expressed in retinal ganglion cells, has been reported to display circadian rhythmicity in mRNA and protein expression (Hannibal, 2006) that may also influence the ERG rhythm, since this rhythm is abolished in mice lacking melanopsin (Barnard et al., 2006). Taken together, ERG response rhythmicity can potentially be modulated by several interacting mechanisms under the direct or indirect control of the circadian clock and could therefore represent a novel method to assess individual circadian phase.

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

REFERENCES

Baba K, Pozdeyev N, Mazzoni F, Contreras-Alcantara S, Liu C, Kasamatsu M, Martinez-Merlos T, Strettoi E, Iuvone PM, Tosini G. (2009). Melatonin modulates visual function and cell viability in the mouse retina via the MT1 melatonin receptor. *Proc. Natl. Acad. Sci. U. S. A.* 106:15043–15048.

Barlow RB Jr. (1983). Circadian rhythms in the Limulus visual system. *J. Neurosci.* 3:856–870.

Barnard AR, Hattar S, Hankins MW, Lucas RJ. (2006). Melanopsin regulates visual processing in the mouse retina. *Curr. Biol.* 16:389–395.

Brainard GC, Lewy AJ, Menaker M, Fredrickson RH, Miller LS, Weleber RG, Cassone V, Hudson D. (1988). Dose-response relationship between light irradiance and suppression of plasma melatonin in human volunteers. *Brain Res.* 454:212–218.

Brandenburg J, Bobbert AC, Eggelmeyer F. (1983). Circadian changes in the response of the rabbits retina to flashes. *Behav. Brain Res.* 7:113–123.

Cameron MA, Lucas RJ. (2009). Influence of the rod photoresponse on light adaptation and circadian rhythmicity in the cone ERG. *Mol. Vis.* 15:2209–2216.

Cameron MA, Barnard AR, Hut RA, Bonnefont X, van der Horst GT, Hankins MW, Lucas RJ. (2008a). Electroretinography of wild-type and Cry mutant mice reveals circadian tuning of photopic and mesopic retinal responses. *J. Biol. Rhythms* 23:489–501.

Cameron MA, Barnard AR, Lucas RJ. (2008b). The electroretinogram as a method for studying circadian rhythms in the mammalian retina. *J. Genet.* 87:459–466.

Cosci B, Longoni B, Marchiafava PL. (1997). Melatonin induces membrane conductance changes in isolated retinal rod receptor cells. *Life Sci.* 60:1885–1889.

Danilenko KV, Cajochen C, Wirz-Justice A. (2003). Is sleep per se a zeitgeber in humans? *J. Biol. Rhythms* 18:170–178.

Danilenko KV, Plisov IL, Wirz-Justice A, Hébert M. (2009). Human retinal light sensitivity and melatonin rhythms following four days in near darkness. *Chronobiol. Int.* 29:93–107.

Dearry A, Barlow RB (1987). Circadian rhythms in the green sunfish retina. *J. Gen. Physiol.* 89:745–770.

Doyle SE, Grace MS, McIvor W, Menaker M. (2002). Circadian rhythms of dopamine in mouse retina: the role of melatonin. *Vis. Neurosci.* 19:593–601.

Emser W, Dechoux R, Weiland M, Wirz-Justice A. (1993). Melatonin decreases the amplitude of the b-wave of the human electroretinogram. *Experientia* 49:686–687.

Gaddy JR, Rollag MD, Brainard GC. (1993). Pupil size regulation of threshold of light-induced melatonin suppression. *J. Clin. Endocrinol. Metab.* 77:1398–1401.

Gagné AM, Danilenko KV, Rosolen SG, Hébert M. (2009). Impact of oral melatonin on the electroretinogram cone response. *J. Circadian Rhythms* 7:14.

Grace MS, Wang LM, Pickard GE, Besharse JC, Menaker M. (1996). The tau mutation shortens the period of rhythmic photoreceptor outer segment disk shedding in the hamster. *Brain Res.* 735:93–100.

Hankins MW, Jones RJM, Ruddock KH. (1998). Diurnal variation in the b-wave implicit time of the human electroretinogram. *Vis. Neurosci.* 15:55–67.

Hannibal J. (2006). Regulation of mealopsin expression. *Chronobiol. Int.* 23:159–166.

Hébert M, Lachapelle P, Dumont M. (1996). Reproducibility of electroretinograms recorded with DTL electrodes. *Doc. Ophthalmol.* 91:333–342.

Manglapus MK, Uchiyama H, Buelow NF, Barlow RB. (1998). Circadian rhythms of rod-cone dominance in the Japanese quail retina. *J. Neurosci.* 18:4775–4784.

Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M; International Society for Clinical Electrophysiology of Vision. (2009). ISCEV standard for full-field clinical electroretinography (2008 update). *Doc. Ophthalmol.* 118:69–77.

Miranda-Anaya M, Bartell PA, Menaker M. (2002). Circadian rhythm of Iguana electroretinogram: the role of dopamine and melatonin. *J. Biol. Rhythms* 17:526–538.

Nozaki S, Wakakura M, Ishikawa S. (1983). Circadian rhythm of human electroretinogram. *Jpn. J. Ophthalmol.* 27:346–352.

Peters JL, Cassone VM. (2005). Melatonin regulates circadian electroretinogram rhythms in a dose- and time-dependent fashion. *J. Pineal Res.* 38:209–215.

Portaluppi F, Smolensky MH, Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. *Chronobiol. Int.* 27:1911–1929.

Remé CE, Wirz-Justice A, Terman M. (1991). The visual input stage of the mammalian circadian pacemaking system: I. Is there a clock in the mammalian eye? *J. Biol. Rhythms* 6:5–29.

Ren JQ, Li L. (2004). A circadian clock regulates the process of ERG b- and d-wave dominance transition in dark-adapted zebrafish. *Vision Res.* 44:2147–2152.

Ribelayga C, Cao Y, Mangel SC. (2008). The circadian clock in the retina controls rod-cone coupling. *Neuron* 59:790–801.

Ruan GX, Allen GC, Yamazaki S, McMahon DG. (2008). An autonomous circadian clock in the inner mouse retina regulated by dopamine and GABA. *PLoS Biol.* 6:e249

Rufiange M, Rousseau S, Dembinska O, Lachapelle P. (2002). Cone-dominated ERG luminance-response function: the Photopic Hill revisited. *Doc. Ophthalmol.* 104:231–248.

Solís-Chagoyán H, Mendoza-Vargas L, Fuentes-Pardo B. (2008). Melatonin modulates the ERG circadian rhythm in crayfish. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 149:373–379.

Storch KF, Paz C, Signorovitch J, Raviola E, Pawlyk B, Li T, Weitz CJ. (2007). Intrinsic circadian clock of the mammalian retina: importance for retinal processing of visual information. *Cell* 130:730–741.

Tosini G, Pozdeyev N, Sakamoto K, Iuvone PM. (2008). The circadian clock system in the mammalian retina. *Bioessays* 30:624–633.

Tuunainen A, Kripke DF, Cress AC, Youngstedt SD. (2001). Retinal circadian rhythms in humans. *Chronobiol. Int.* 18:957–971.

Van de Werken M, Gimenez MC, Van Nierop LE, De Vries B, Beersma DGM, Gordijn MCM. (2009). Simulated night shift work under white, yellow and dim light. *In EBRS Programme & Abstract Book*, p. 204. XI Congress of the European Biological Rhythms Society, Strasbourg, France, August 22-28, 2009.

Verde MA, Barriga-Montoya C, Fuentes-Pardo B. (2007). Pigment dispersing hormone generates a circadian response to light in the crayfish, *Procambarus clarkii*. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 147:983–992.

Wachtmeister L. (1998). Oscillatory potentials in the retina: what do they reveal. *Prog. Retin. Eye Res.* 17:485–521.

Weber JM, Schwander JC, Unger I, Meier D. (1997). A direct ultrasensitive RIA for the determination of melatonin in human saliva: comparison with serum levels. *Sleep Res.* 26:757.

White MP, Hock PA. (1992). Effects of continuous darkness on ERG correlates of disc shedding in rabbit retina. *Exp. Eye Res.* 54:173–180.

Wirz-Justice A, Da Prada M, Remé C. (1984). Circadian rhythm in rat retinal dopamine. *Neurosci. Lett.* 45:21–25.

Wu WQ, McGoogan JM, Cassone VM. (2000). Circadian regulation of visually evoked potentials in the domestic pigeon, *Columba livia*. *J. Biol. Rhythms* 15:317–328.

Zeitzer JM, Dijk DJ, Kronauer RE, Brown EN, Czeisler CA. (2000). Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. *J. Physiol. (Lond.)* 526:695–702.