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SHORT COMMUNICATION

AGE-DEPENDENT ALTERATIONS IN HUMAN *PER2* LEVELS AFTER EARLY MORNING BLUE LIGHT EXPOSURE

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In our modern society, we are exposed to different artificial light sources that could potentially lead to disturbances of circadian rhythms and, hence, represent a risk for health and welfare. Investigating the acute impact of light on clock-gene expression may thus help us to better understand the mechanisms underlying disorders rooted in the circadian system. Here, we show an overall significant reduction in *PER2* expression in oral mucosa with aging in the morning, noon, and afternoon. In the afternoon, 10 h after exposure to early morning blue light, *PER2* was significantly elevated in the young compared to green light exposure and to older participants. Our findings demonstrate that human buccal samples are a valuable tool for studying clock-gene rhythms and the response of *PER2* to light. Additionally, our results indicate that the influence of light on clock-gene expression in humans is altered with age. (Author correspondence: urs.albrecht@unifr.ch)

Keywords *PER2*, Monochromatic light, Age, Wavelength, Human circadian rhythms, Oral mucosa

INTRODUCTION

Humans and other mammals receive light information affecting the circadian system mainly via a subset of non-visual intrinsically photosensitive retinal ganglion cells (ipRGC) situated in the inner retina (Berson

et al., 2002; Moore et al., 1995; Provencio et al., 2000; Sekaran et al., 2003). These cells express the blue light-sensitive photopigment melanopsin (Gooley et al., 2001; Hattar et al., 2002; Lucas et al., 2001; Provencio et al., 2000) and send photic information directly to the suprachiasmatic nuclei (SCN) via the retino-hypothalamic tract (RHT) (Moore & Lenn, 1972). Because exposure to polychromatic and monochromatic short-wavelength light has a rather widespread impact on human behavior, ranging from subjective mood to brain wave activity, disturbances in the ipRGC photoreception could lead to physiological and psychological problems (Buijs et al., 2006; Klerman, 2005). For instance, blue light exposure acutely increases core body temperature, heart rate (Cajochen et al., 2005), alertness (Cajochen et al., 2005; Lockley et al., 2006), and cognition (Vandewalle et al., 2007). However, light does not only influence variables downstream from the central circadian clock but also affects its underlying molecular mechanisms. For example, a nocturnal light pulse differentially induces the clock genes *Per1* and *Per2* in mice (Albrecht et al., 1997; Yan & Silver, 2002), and in humans, evening blue light can induce expression of *PER2* in oral mucosa (Cajochen et al., 2006). Here, we aimed to investigate whether morning light exposure is also capable of influencing *PER2* levels in human oral mucosa and whether such a change is wavelength- or age-dependent.

MATERIALS AND METHODS

Study Design and Processing of Human Oral Mucosa

The study has been conducted as previously described by Sletten et al. (2009). In brief, 11 young (23.0 ± 2.9 yrs, mean $\pm$ SEM) and 15 older (65.8 ± 5.0 yrs) healthy males participated in the study. All subjects were free from medical, psychiatric, and sleep disorders. An ophthalmologic examination was carried out prior to the study to ensure the volunteers were free of ocular disorders (e.g., color blindness, glaucoma, severe cataracts). The study was approved by the University of Surrey Ethics Committee, and all participants gave written informed consent before the study began. All procedures were conducted in accordance with the Declaration of Helsinki and conformed to international ethical standards (Portaluppi et al., 2008).

The participants received a 2 h intermittent monochromatic light exposure 8.5 h after their individual dim light melatonin onset (DLMO). This 2 h time window for the light exposure was chosen based on the human phase-response curve, which shows maximal phase advances 7 to 9 h after the DLMO (Revell & Eastman, 2005). Subjects received blue (λ_{max} 456 nm) or green (λ_{max} 548 nm) light matched for photon density (6×10^{13} photons/cm^2/s) on separate occasions. Identical photon densities were used because photopigments act as photon counters. Hence,
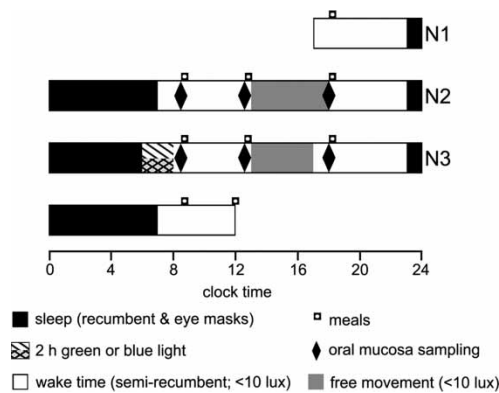
FIGURE 1 Overview of the protocol design. The figure illustrates an example protocol for a participant maintaining a 23:00–07:00 h sleep schedule with light and buccal sampling timed according to a DLMO at 21:00 h. Subjects had to maintain a consistent, self-selected sleep schedule seven days prior to and throughout the study. In the laboratory, the volunteers took part in a baseline phase-assessment followed by an experimental light session when they were subjected to 2 h of either blue (single-hatched) or green (cross-hatched) light in the early morning. Oral mucosa was sampled at identical time points on the baseline day (between night 1 [N1] and N2) and 0.5, 5, and 10 h after the light administration (between N2 and N3). The entire protocol was carried out under controlled light levels, posture, and meals.

different monochromatic light stimuli can only be compared if they contain an equal number of photons (Albrecht & Foster, 2002).

Eight subjects had four weeks between light conditions, and 13 had two weeks between conditions. All young subjects were exposed to both wavelengths, whereas 5 older subjects were exposed to blue light only. Oral mucosa was sampled at clock times equivalent to 0.5, 5, and 10 h after light exposure on a baseline day without light exposure and 24 h later on the day of light exposure (see Figure 1). The collection of human oral mucosa and the subsequent RNA isolation, cDNA synthesis, and quantitative real-time PCR for *GAPDH* and *PER2* were performed as described previously (Cajochen et al., 2006). Some samples were lost from analysis, as *PER2* levels could not be determined in every sample due to insufficient amounts of mRNA (analyzed: young N = 6–10; old N = 7–13 for both green and blue light).

Data Analysis

The $2^{\Delta CT}$ value per mucosa sample was calculated according to the following formula (*a, b*, and *c* correspond to replicate 1, 2 and 3):

$$2^{\Delta CT} = (2^{GAPDHa-PER2a} + 2^{GAPDHa-PER2b} + 2^{GAPDHa-PER2c}$$
$$+ 2^{GAPDHb-PER2a} + 2^{GAPDHb-PER2b} + 2^{GAPDHb-PER2c}$$
$$+ 2^{GAPDHc-PER2a} + 2^{GAPDHc-PER2b} + 2^{GAPDHc-PER2c})/9$$

For statistical analysis, the mean $2^{\Delta CT}$ value was used for every subject and log-transformed to attain a normal distribution. Mixed-model analyses of variance for repeated measures, rANOVA (PROC mixed), with factors of age (young and older), treatment (blue light, green light, no-light) and time (0.5, 5, and 10 h after light exposure) was performed. Subsequently, separate rANOVAs for the young and older group were performed with the factors of treatment and time. Contrasts were assessed with the LSMEANS statement, and all p values for the r-ANOVA were based on the Kenward-Roger's corrected degrees of freedom. The Tukey-Kramer test for unbalanced data was utilized in the PROC mixed to adjust for post-hoc multiple comparisons. This mixed-model regression analysis approach is appropriate to address large inter-individual differences within the data set and to take into account the correlations between the numerous observations collected from each subject. Thus, this approach properly addresses within- and between-subjects variance (VanDongen, 2004). The statistical package SAS® (SAS Institute Inc., Cary, North Carolina, USA; Version 6.12) was used for all data analyses.

RESULTS

A rANOVA with factors of age, treatment, and time yielded significances for the main effects of age [$F(1, 19) = 9.4$, $p < 0.007$] and time [rANOVA, $F(2, 173) = 8.6$, $p < 0.0004$], indicating higher *PER2* expression levels in the young compared to the older participants and a significant time of day effect. Separate rANOVAs for the younger subjects revealed significant time effects [$F(1, 77) = 5.7$, $p < 0.005$] and a tendency for the interaction treatment and time [$F(1, 76)=2.1$, $p=0.06$]. Post-hoc comparisons revealed that *PER2* expression levels were significantly increased in young subjects 10 h after blue (t = -2.09; $p < 0.04$) but not green light compared to the no-light condition (see Figures 2A and 2B). By contrast, neither blue nor green light had an effect on *PER2* expression in oral mucosa samples of older subjects (see Figures 2C and 2D), except for the factor of time; all other factors did not yield significance, with p at least 0.2. Thus, no post-hoc comparisons were performed for the older participants.

DISCUSSION

Early morning short-wavelength light in the blue range (456 nm) did not induce an immediate alteration in *PER2* expression in human oral mucosa, as reported with a similar blue light 2 h exposure in the evening after 0.5 h (Cajochen et al., 2006), but a rather delayed (10 h) increase in *PER2* expression in comparison to the no-light condition (see Figures 2A and 2C). This time-dependent acute inducibility of human
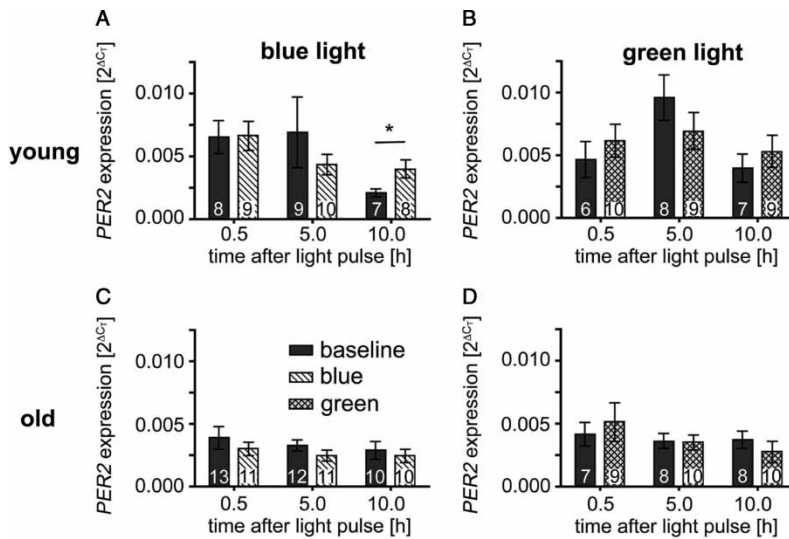
FIGURE 2 Age- and wavelength-dependent changes in *PER2* expression in human oral mucosa. Young and old subjects were exposed to either 2 h of monochromatic blue (λ_{max} 456 nm) or green (λ_{max} 548 nm) light. *PER2* expression was measured using quantitative real-time PCR. Data are represented as mean $\pm$ SEM; N for each condition and time point is indicated at the bottom of the respective bars. Samples taken after the light pulse were compared to the matching baseline samples (taken between N1 and N2). *$p < 0.04$.

PER2 is in line with previous observations in mice where *Per2* is acutely induced in the early dark phase but poorly in the late dark phase (Albrecht et al., 1997; Yan & Silver, 2002). In contrast to blue light, exposure to green light did not produce any change in *PER2* expression (see Figures 2B and 2D). This wavelength dependence of *PER2* gene expression is consistent with previous findings (Cajochen et al., 2006).

In our study, the effect of blue light on *PER2* levels was age-dependent: only in young subjects was a significant effect of blue light demonstrated. The significant difference of *PER2* levels in young subjects 10 h after the blue-light pulse versus no light is likely due to a phase shift of the mRNA expression curve (see Figure 2A). However, one has to be cautious with this interpretation, as the entire 24 h profile of *PER2* expression was not assessed, and the increase in *PER2* expression due to blue light was only significant in the young and not the older cohort. Furthermore, our data do not provide information about the direction of the shift. Nevertheless, data from the same subjects indicate that early morning blue light provoked a phase advance in the circadian melatonin profile in the plasma (Sletten et al., 2009). Interestingly, evening blue light not only caused an acute induction of *PER2* in oral mucosa, but it also appears that *PER2* levels tended to be lower compared to the no-light control 10 h after light administration the following morning (Cajochen et al., 2006). This indicates a potential phase delay due to evening blue light. Taken

together, it appears that light has comparable effects on human *PER2* mRNA levels as previously observed in mice. Furthermore, our results in humans indicate that aging impairs short-wavelength, light-activated responses impinging on the circadian system (see Figure 2C), as previously observed in hamsters (Zhang et al., 1996) and humans (Herljevic et al., 2005; Sletten et al., 2009). An age-related reduction in amplitude has also been reported previously for circadian rhythms in temperature and sleep (Carrier et al., 1996; Monk et al., 1995). Moreover, a significant reduction in the amplitude of the *PER3* rhythm in human leukocytes has been observed in older people in the current data set (Ackermann et al., 2009). With age, the crystalline lens absorbs more light (Pokorny et al., 1987; Turner & Mainster, 2008; Weale, 1985), and the pupil area decreases (Verriest, 1971; Yang et al., 2002); thus, blue light transmission is reduced. This could result in impaired circadian photoreception (Herljevic et al., 2005) and be partly responsible for the changes observed in the sleep-wake cycle and circadian rhythmicity in the elderly (Bliwise, 1993; Duffy et al., 1998).

Although our results suggest that blue light in the morning leads to an increase in *PER2*, the small number of subjects and the few sampling points do not allow definite conclusions about the effect of morning light on *PER2* expression. Further studies measuring more time points and including more subjects are needed to better understand how clock-gene expression is influenced by light in the human oral mucosa or other peripheral clocks. Nonetheless, the current results highlight similarities in the light response of the circadian system between rodents and humans. In addition, our report illustrates that buccal samples are a valuable tool for studying the response of clock genes to light in humans.

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