Human Melatonin Suppression in Response to Silent Substitution Stimuli of Photoreceptors

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ABSTRACT

It is important to understand how photoreceptors contribute to non-image-forming visual functions to enable the design of light environments that support optimal circadian rhythm regulation. The purpose of this study was to investigate the contribution of melanopsin-expressing ganglion cells (mRGCs) to circadian rhythm regulation using the silent substitution method, which modulates light stimulus levels to each photoreceptor (mRGC and three types of cones). Night-time saliva melatonin concentrations were measured to assess its contribution to circadian rhythms. We found that melatonin suppression was significantly affected by mRGC stimulation which was modulated by the silent substitution method, especially at time early in melatonin secretion.

KEYWORDS: melanopsin-expressing retinal ganglion cell, melatonin, silent substitution method

1. Introduction

The discovery of a novel photoreceptor, the melanopsin-expressing retinal ganglion cell (mRGC) early in the 21st century, has raised questions about its role in photoreceptive tasks. Particularly of interest has been how mRGCs compare with rods and cones, especially in non-image-forming visual functions, such as circadian rhythm regulation, the pupillary light reflex, and activity of the autonomous nervous system and arousal levels.1-5

Melatonin secretion and its suppression by night-time light exposure are often considered to be an indicator of circadian rhythm regulation in humans6-7. Melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted mainly by the pineal gland in the brain, and its secretion is controlled by the suprachiasmatic nucleus (SCN) in the hypothalamus, which functions as the central biological clock responsible for circadian rhythms. Melatonin secretion usually peaks during the night and is suppressed by exposure to light at night (LAN). The temporal relationship between the temperature nadir, sleepiness, and the peak of melatonin excretion has been proven experimentally8. It has been reported that the amount of melatonin suppression depends not only on the light intensity but also on its spectrum9,10. Melatonin suppression is the most sensitive to light with short wavelengths (about 460nm, in the blue part of the spectrum), which this is close to the peak sensitivity (about 480nm) of the mRGCs to light. The German Institute for Standardization (DIN) and the Lighting Research Center in the US11 have proposed methods to calculate the impact of light on melatonin suppression based on the spectral distribution of the light and spectral sensitivity of each type of the photoreceptors (rods, three types of cones, and mRGCs).

It is important to understand how these photoreceptors contribute to non-image-forming visual functions to enable the design of light environments that support optimal circadian rhythm regulation. Although mRGCs are known to be important photoreceptors for circadian rhythm regulation, cones may also have a role. It has been suggested that cones contribute to non-visual responses, such as melatonin suppression and phase shift at an early time of light exposure and low irradiance, while mRGCs appear to be the primary photoreceptor in response to long-duration light exposure and high irradiance12. However, the contribution of mRGCs and cones to non-visual responses may be more complicated since mRGCs receive synaptic input from rods and cones in addition to having an intrinsic photoreceptor in intact retinae13-15. To elucidate how mRGCs function in the non-visual system, mRGC responses should be produced and measured independently of cone and rod responses. Silent substitution is a method which enables us to modulate stimulus levels to mRGCs and cones independently, while keeping the same color and illuminance16-18. Previously, we investigated responses to light stimuli using the method and responses to the stimulus were successfully recorded with an electroretinogram19,20 and a pupil monitor21,22.
This study aimed to investigate the contributions of mRGCs to circadian rhythm regulation in humans by using the silent substitution method, which modulates stimulus levels to each photoreceptor. Night-time saliva melatonin concentrations were measured as a way to assess the contributions of the mRGCs.

2. Methods

2.1 Apparatus and light condition

The illumination system was custom-built (Fukuoka Women’s University, Japan) to achieve the receptor-silent substitution\textsuperscript{29}. Four-in-one light-emitting diodes (LEDs) at peak wavelengths of 460 nm (blue), 525 nm (green), 635 nm (red), and 445/555 nm (white) with corresponding half-height bandwidths of 24, 40, and 22 nm were used to build a projector. The circular light stimulus was projected onto a diffuser which was 300 mm from a participant, and the circle subtended a visual angle of about 19°. The luminance output of each LED was controlled by a controller. A spectroradiometer (LightSpex, GretagMacbeth Co., Switzerland) was used to measure the light stimuli to confirm the stimulus levels. Stimulus levels ($\alpha$-opic lx) for each photoreceptor (S-cone, M-cone, L-cone, and mRGC) were calculated from the spectral power distribution of the light and $\alpha$-opic sensitivity curves of short (S), middle (M), and long (L) wavelength-sensitive cones and the mRGC\textsuperscript{29}. Table 1 shows $\alpha$-opic lx provided to the photoreceptors under the three light conditions (Control, mRGC, and 1.4 energy) and the dim light condition (Dim). In the mRGC condition, the stimulus intensity was increased by approximately 40% from the control, whereas the intensity was not changed for the cones. In the 1.4 energy condition, the stimulus intensity for all photoreceptors (mRGC and three types of cones) was increased by approximately 40% from the control. The rod stimulus was not calculated as the stimulus level $>1000$ lx was considered as reaching photopic vision (the level at which rods are saturated).

2.2 Experimental procedure

Fifteen female Japanese students (21–23 years old) from Fukuoka Women’s University completed the experiments. The experiment was performed over four days that included dim light conditions ($<50$ lx) in a laboratory at Fukuoka Women’s University in Japan from September to December 2015. The air temperature and relative humidity were set at 26°C and 60% RH, respectively. For one week before the experiment, participants were asked to maintain a regular sleep-wake cycle (sleeping at 00:00 h and waking at 07:00 h). For each participant, one of the four conditions was randomly chosen and performed in a day. Most of the interval between the experiments was 4–6 days (for thirteen participants), while only a day for two partici-

| Table 1  | Stimulus levels ($\alpha$-opic lx) to the cones and mRGC. |
|----------|--------------------------------------------------------|
|          | S-cone | M-cone | L-cone | mRGC | Illuminance (lx) |
| Control  | 188.1 (100) | 189.4 (100) | 188.7 (100) | 154.3 (100) | 1000 |
| mRGC     | 192.3 (102) | 197.8 (104) | 198.0 (105) | 225.0 (146) | 1000 |
| 1.4 energy | 263.4 (140) | 265.1 (140) | 264.2 (140) | 216.0 (140) | 1400 |
| Dim      | —      | —      | —      | —      | $<50$ lx |

Relative stimulus levels are shown in brackets.

Figure 1 Clinical protocol.

2.3 Analysis method

Saliva samples were analyzed with RIA kits (RK-DSM2 200 tests, Bühlmann Laboratories AG, Switzerland). The mean coefficients of variation of the intra- and inter-assay precision were 7.9% and 9.8%, respectively. The limit of detection was 0.2 pg/mL and the limit of quantification was 0.9 pg/mL. All saliva samples for a given participant were analyzed on the same RIA plate. The melatonin data from twelve participants were statistically analyzed as their melatonin secretion was successfully detected. All participants had already started melatonin secretion at the beginning of the saliva sampling at 22:30 and the data of melatonin secretion was not normally distributed. Therefore, to account for individual differences and non-normal distribution of the data, melatonin concentrations were converted to ratios using the Eq. (1). Then, the percentage of melatonin suppression (relative to dim light) at 23:00–00:00 h
was calculated by the Eq. (2). After exclusion of an outlier using the Grubbs’ test, eleven participants’ data were used for comparisons of light effects. The normality of the data about melatonin suppression was confirmed using the Shapiro–Wilks normality test and the normal Q–Q plots.

A two-way ANOVA for repeated measurements of Condition (Control, mRGC, and 1.4 energy) and Time (23:00, 23:30 and 00:00) was performed to examine melatonin suppression. In addition, the area under the curve (AUC) during the 1.5-h light exposure was calculated with the trapezoidal method to determine the overall effect of light exposure on melatonin suppression. The data was normally distributed. The effect of Condition on melatonin suppression at each time and AUCs of melatonin suppression were analyzed using one-way ANOVA. The Bonferroni method was used to correct for multiple comparisons using ANOVA, and the threshold p value for individual tests was 0.05/3=0.016. All analyses were conducted in SPSS (ver. 22; IBM Co., Japan), and p<0.05 was considered to be statistically significant. The data in all graphs are presented as means±SD (standard deviation).

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\text{Melatonin ratio concentration} = \frac{\text{concentrations at times 23:00–00:00h}}{\text{concentration at 22:30h}} \tag{1}
\]

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\%\text{ melatonin suppression} = \left( \frac{D-L}{D} \right) \times 100 \tag{2}
\]

D: Melatonin ratio concentration under dim light condition
L: Melatonin ratio concentration under a light condition

3. Results and Discussion

Figure 2 shows mean saliva melatonin concentration of eleven participants. Melatonin secretion was suppressed under the three light conditions—Control, mRGC, and 1.4 energy. Figure 3 shows the amount of melatonin suppression (%) under the three conditions. There was no significant interaction between Condition and Time. Melatonin suppression tended to be differed by Condition (F(2, 20)=3.2, p=0.06) and Time (F(2, 20)=3.3, p=0.06), with melatonin suppression was higher at 00:00h than at 23:00h, while such differences were not found for multiple comparisons of the light conditions. Furthermore, melatonin suppression differed significantly in the three light conditions at time early in melatonin secretion. At 23:00h, melatonin suppression was significantly different among the three light conditions (F(2, 20)=5.1, p<0.05), with the tendency of increased melatonin suppression in the mRGC condition compared with the control (p=0.1). There was a tendency of differed melatonin suppression among the three light conditions at 23:30h (F(2, 20)=2.5, p=0.1), while no significant difference among them at 00:00h. These results suggested that the contribution of mRGs to light-induced melatonin suppression might depend on duration of the light exposure. Overall, the results demonstrate that melatonin secretion differed in the three light conditions that accounted for modulation of photoreceptor stimulation by the silent substitution method, especially at time early in melatonin secretion. The AUCs were calculated to determine the overall effect of light exposure on melatonin suppression (Figure 4). Condition had a significant effect on AUC (F(2, 20)=3.7, p<0.05), with tendency of increased AUC in the mRGC condition compared with the control (p=0.1). Although illuminance of light exposure in the 1.4 energy condition was 1.4 times higher than in the mRGC condition, there was no significant difference between the 1.4 energy condition and the control.

This study demonstrated that circadian rhythm regulation was primarily due to the effect of mRGs and that increased energy did not have significant effects. However, we did not cover a wide range of illuminance, nor did we examine melatonin concentration in response to cone stimulation alone. A previous study suggested that the cone contributes to melatonin suppression and circadian phase resetting under short-duration.
light exposure and low irradiance\(^{12}\). However, in their method, blue (460 nm) and green (535 nm) light exposure stimulated both the cones and mRGCs as their sensitivity curves overlap\(^{20}\). Our method enables independent stimulation of mRGCs and cones, and this technique is potentially useful for further studies on how cones and mRGCs participate in non-image-forming visual functions. Since a number of studies have suggested that circadian rhythm disruption has harmful effects on human health\(^{15-39}\), further studies with detailed observations of how cones and mRGCs contribute to circadian rhythm regulation are required.

There are some limitations of this study. The effects of LAN on individuals differ according to their light history\(^\text{31}\). Two participants experienced an interval of a day; it might cause differences on response to subsequent light condition, although we did not observe any effects of the short-term interval on the results. Therefore, additional regulation of interval, daytime light exposure and activity may be required to compare the effects of our test stimuli. In addition, the concentration of plasma melatonin is age-\(^{32}\), race-\(^{33}\), and ocular condition-dependent\(^{34,35}\). Therefore, the effects of light on melatonin secretion will differ between individuals, and such individual differences may affect the impact of light exposure on human health. Even with these limitations, our findings may have important implications for the development of light environments and optimization of light use for preventing circadian rhythm disruption.

4. Conclusions

Melatonin suppression differed significantly at time early in melatonin secretion under the three light conditions that accounted for modulation of photoreceptor stimulation by silent substitution method. Further, melatonin suppression was mostly affected by mRGC stimulation but not by increased energy. The findings may have important implications for optimizing the light use and designing light environments for preventing circadian rhythm disruption.

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