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Effects of Red Light on Circadian Rhythm: A Comparison Among Lamps With Similar Correlated Color Temperatures Yet Distinct Spectrums

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ABSTRACT Blue crest between the wavelength of 460 nm and 480 nm was reported to present melatonin suppression effects, whereas effects of red light on circadian rhythm regulation remain unclear. Spectrum plays an important role in circadian rhythm regulation, yet a lot of researches focused on the correlated color temperature, although a correlated color temperature value corresponds to various possible spectrums. Here, we performed human factor experiments with 3 lamps on 17 participants, comprising 9 males and 8 females. Our results showed that spectrums with high blue intensity tended to cause abnormal regulations of melatonin and cortisol, while the abnormalities were likely to be compensated by the 606-635-nm red light, which was indispensable for the photo-biological effects concerning circadian rhythm regulation. Abnormal circadian rhythm regulation was also found to be influenced by the illuminance, as abnormalities were significant in 500 lux whereas they were likely to disappear in 250 lux, implying the existence of threshold doses to trigger abnormities concerning circadian rhythm regulation. Furthermore, circadian rhythm responses were distinct between males and females. Our work may have implications for the development of light source, as we suggest that lighting source designers should increase the 606-635-nm intensity for bed room luminaires to decrease melatonin suppression effects.

INDEX TERMS Spectral power distribution, circadian rhythm regulation, narrow blue crest, gender difference, long-wavelength red light.

I. INTRODUCTION

Ambient light affects visual comfort [1], p2] and ocular safety [3], [4] through a series of neural and chemical processes. The characterization of ambient light is described by a set of photometric parameters, which correspond to the featured photo-biological (visual and non-visual) effects [5]–[7]. Concerning the health-related issues caused by the ambient light, standards have listed detailed requirements on the photometric parameters [8]. Light source generally comprises LED and phosphor materials, and the quality and arrangement of the light source determines the optical performance of lamp and display [9]–[11]. Light source development is accelerated by the abundant knowledge of photo-biological effects [12]–[14], therefore studies on related issues are necessary and pressing.

Light intensity is a significant photometric parameter due to the relation to power, and it is generally quantified as illuminance or brightness [15], [16], or the sum of photon energy [16]. Light color is determined by the wavelength distribution (also known as the spectral power distribution) and also related to the visual and non-visual effects [17], [18]. Visual effects are generally reflected by ocular structural variations [19], while non-visual effects are usually characterized

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by neural and hormone responses [20]. Melatonin is an extensively used indicator for non-visual effects regarding circadian rhythm regulation, as the melatonin content presents periodic fluctuation with clock time [21]–[23]. Normal melatonin content is necessary in sleeping, immune function and cardiovascular activities [22], [23]. Researches on light-intervened melatonin emission have reported the suppression effects of the high-blue-intensity spectrum [24]–[26].

Photo-biological effects have been reported to be affected by correlated color temperature (CCT) in a number of studies [27]–[30], whereas the fact that a CCT corresponds to multiple SPDs tend to be ignored in previous researches [31], [32], resulting to the lack of requirements regarding spectrum in available standards for lamps and displays. In the fabrication of light source, the mixture of red, green and blue phosphors contributes to the final color [33]–[35], therefore a certain color corresponds to various possible phosphor proportions and photoluminescence spectrums [31]. Lacking in the knowledge on the photo-biological effects of spectrum has limited the health-related development of lighting and displaying.

In the current study, we investigated 3 lamps with similar power and CCTs (around 5000 K) yet distinct spectrums, by human factor experiments on 17 participants. We analyzed the characterizations of melatonin and cortisol emission following the use of each lamp. We were sought to clarify the effects of similar-CCTs-distinct-SPDs light on circadian rhythm regulation, especially the non-visual effects of red light on circadian rhythm regulation.

II. TESTING AND MEASUREMENT METHOD

A. AMBIENT LIGHT

The light sources used in our experiments comprised a dorm lamp (4000 K) and 3 experimenting lamps (around 5000 K). The dorm lamp was installed as the default lighting device of the student dorm, and participants had been accustomed to the dorm lamp as the daily lighting. The dorm lamp was used as the comparison baseline, while the 3 experimenting lamps were used in our experiments for comparison. The lamps presented distinct spectrums (Fig.1), although other optical performances were similar (Table 1). Here, the CCT value of each experimenting lamp was around 5000 K, as suggested in standards and previous literatures [8], [36].

B. SUBJECTS

In the present study (including the experimental protocols), all of the 17 participants (university students, including 9 males and 8 females, with the age from 21 to 30 years) provided written informed consent, and all of the methods used were performed in accordance with the relevant guidelines and regulations. Fundus inspection and blood test were performed on all the subjects to confirm they were appropriate in our experiments. No one had the habit of alcohol, sleeping pills, caffeine or hormone-related drugs. Each participant was required to adapt the sleeping schedule to 22:30-6:30 around 7 days ahead of the experiments with the lighting of dorm lamp.

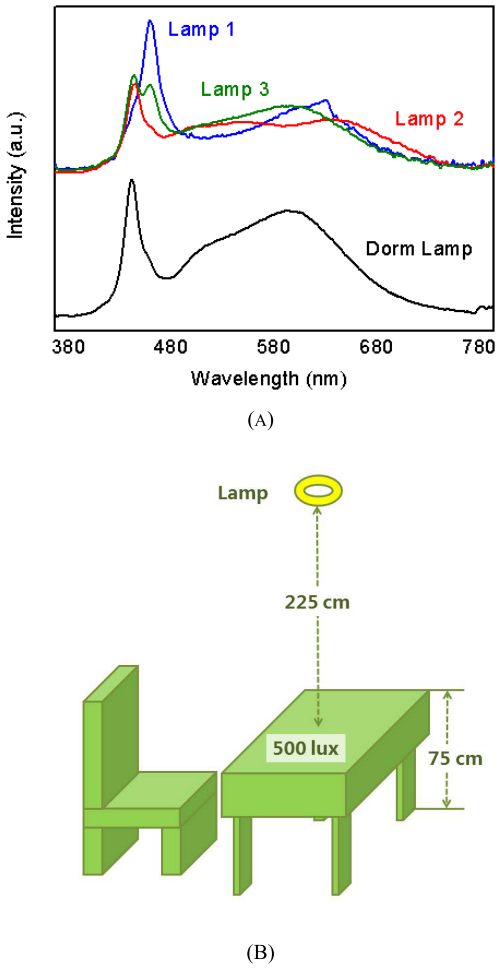

FIGURE 1. Light environments of the experiments: (a) SPD characterization of each lamp, with the total intensity (representing the power) of each lamp normalized to the same value; (b) lamp distribution.

TABLE 1. Optical performance of each lamp.

Lamp ID	CCT (K)	Blue Peak	Ra
Dorm Lamp	4000	450	>80
Lamp 1	5150	453	
Lamp 2	5000	467	
Lamp 3	5050	453 & 467	

C. EXPERIMENTS

Our study lasted from May to July and comprised 2 parts: the visual task part (19:00-22:30, with each participant seated by the desk for printed-materials reading; any screen watching was not allowed) and the sleeping part (22:30-6:30, with all lamps turned off to keep dark). Participants finished supper before 17:30 and then brushed teeth without toothpaste. Eating was not allowed after 18:00. From 18:40 each participant entered his/her own apartment dorm for the

corresponding experiment. The visual task part was over at 22:00, and the corresponding lamp was turned off at 22:30. Participants were required to go to bed prior to 23:00.

During the experiments, the ambient light for each participant was constructed in his/her own room in the apartment dorm. All window curtains were closed to screen out the light outside the room. The lamp was placed 225 cm above the desk, which was 75 cm in height. The lamp power was set to have the illuminance of 500 ± 50 lux on the desk center. Each experimenting lamp was employed for 4 consecutive evenings from Monday to Thursday, while it was replaced by the dorm lamp in the evenings from Friday to Sunday for the recovery of circadian rhythm regulation which was affected by the experimenting lamp. It was considered that 3 days was competent to eliminate the residual effects of the previous experimenting lamp. All the 3 lamps were employed with the desk illuminance of 500 ± 50 lux, and additional experiments using Lamp 1 and Lamp 3 were performed with the desk illuminance of 250 ± 50 lux as they presented abnormal effects. The illuminance 250 ± 50 lux is close to 300 lux, which is the threshold illuminance for reading in hospital ward (GB/T 26189-2010) and is considered to be harmless to participants' visual health within the duration of the visual task. The final rounds of experiments on different lamps were as follows: Lamp 1: 500 ± 50 lux, 250 ± 50 lux; Lamp 2: 500 ± 50 lux; Lamp 3: 500 ± 50 lux, 250 ± 50 lux.

Saliva was collected at 19:00, 22:00, 23:00 and 7:00 every day. During the extraction of melatonin and cortisol from saliva, each participant gargled with pure water and then kept a piece of cotton under the tongue. After 1 min the cotton was extracted from the mouth with tweezers and stored at −20 oC with the SARSTEDT Salivette tube (Germany) for Enzyme-Linked ImmunoSorbent Assay (ELISA) analysis that was used to analyze the levels of melatonin and cortisol. For the ELISA analysis, coefficient of variation inside BRK1519H1/H2 kit was below 10%, and the measurement was performed using the VersaMax microplate reader (450 nm).

III. RESULTS AND DISCUSSION

Circadian rhythm regulation is affected by the ambient light due to the non-visual effects [24]. The biological oscillating clock in mammal generates and regulates the physiological rhythms, which oscillates with a period close to 24 hours, due to the function of the suprachiasmatic nuclei (SCN) locating at the brain's hypothalamus region [24], [25]. Melatonin and cortisol are significant indicators for circadian rhythm, and their content variation with clock time was generally employed to reflect the non-visual effects of ambient light.

Prior to experiments with the 3 experimenting lamps, each participant spent 7 days to adapt to the 22:30-6:30-sleeping schedule. The dorm lamp, which was installed in the ceiling of apartment dorm for daily lighting, was used for the lighting during the 7-day's adaptation as the circadian rhythm baseline. Saliva was collected in the 7th evening from 19:00 to 7:00. Melatonin variations presented the rising trend (females: $t = 5.493$, $p = 0.001$; males: $t = 3.594$, $p = 0.007$) from 19:00 to 23:00, and the decreasing trend (females: $t = 7.42$, $p = 0$; males: $t = 7.055$, $p = 0$) from 23:00 to 7:00. Cortisol variations presented the decreasing trend (females: $t = 12.492$, $p = 0$; males: $t = 7.15$, $p = 0$) from 19:00 to 23:00, and the rising trend (females: $t = 9.376$, $p = 0$; males: $t = 9.953$, $p = 0$) from 23:00 to 7:00 (Fig.2). Melatonin difference between 22:00 and 23:00 was insignificant (females: $t = 0.538$, $p = 0.607$; males: $t = 1.11$, $p = 0.299$). Cortisol difference between 22:00 and 23:00 was insignificant for females ($t = 0.863$, $p = 0.417$) yet significant for males ($t = 2.175$, $p = 0.061$).

Experiments with the 3 lamps were performed following the 7-day's adaptation. Participants presented similar melatonin-variation trends characterized by the rising-to-decreasing features during 19:00-7:00 from Monday to Thursday, except for those using Lamp 1 and Lamp 3 on Wednesday and Thursday (Fig.3). For participants using Lamp 1 and Lamp 3 with the illuminance of 500 lux, melatonin suppression began to appear in the 3rd evening at 22:00 although the melatonin content soared to high level at 23:00. In the 4th evening, the suppression was more significant as the melatonin content at 23:00 was still abnormally low.

With the illuminance decreased from 500 lux to 250 lux, abnormalities regarding melatonin emission disappeared among females using Lamp 1 and Lamp 3, as well as males using Lamp 1. However, males employing Lamp 3 still presented melatonin suppression at 22:00 and 23:00, and melatonin enhancement at 7:00. It was suggested that males were likely to be more sensitive to Lamp 3 in low illuminance compared with females.

Cortisol regulation is related to melatonin content, therefore the cortisol variation with clock time is generally on the contrary to melatonin variation except for the case of continuous sleeping deprivation. In the current study, participants using the 3 lamps presented descending-to-rising trends of cortisol variations during 19:00-7:00 from Monday to Thursday. Similarly, cortisol variations were abnormal for those who used Lamp 1 and Lamp 3 on Wednesday and Thursday (Fig.4).

For participants using Lamp 1 and Lamp 3 in 500 lux, cortisol enhancement began to appear at 22:00 in the 3rd evening whereas the cortisol content dropped to low level at 23:00. In the 4th evening, the cortisol enhancement continued at 23:00. With the illuminance decreased from 500 lux to 250 lux, abnormalities regarding cortisol variation still existed for males using Lamp 3, while all abnormalities disappeared among males using Lamp 1 and females using Lamp 1 and Lamp 3.

As the baseline for melatonin and cortisol variations in our experiments, the dorm lamp was characterized by the spectrum comprising the blue crest at the wavelength of 450 nm, and the correlated color temperature of around 4000 K. Participants using Lamp 2 presented similar

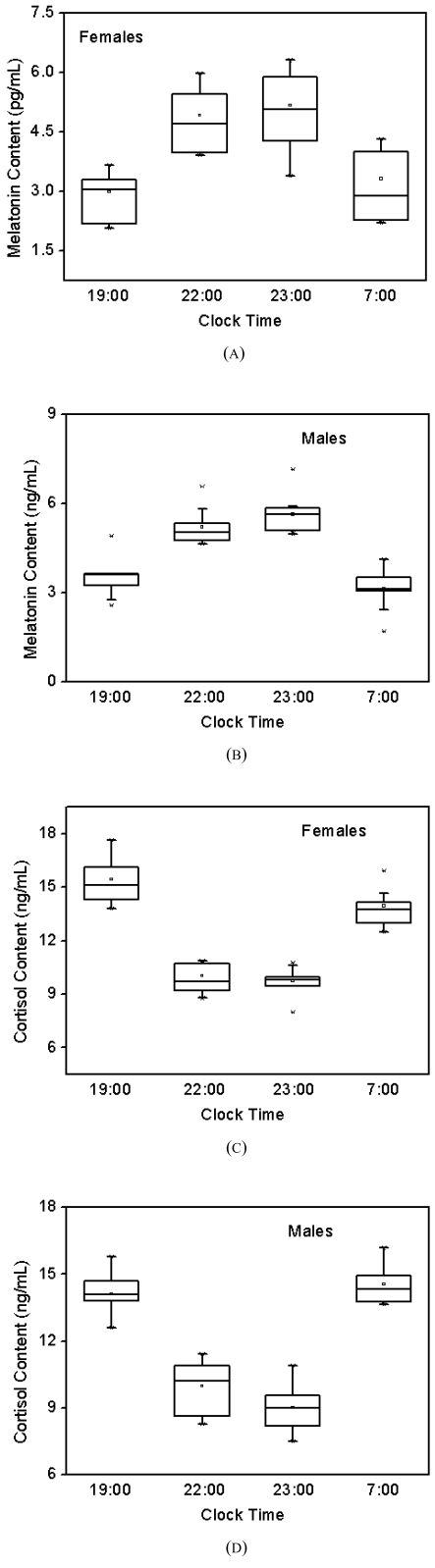

FIGURE 2. Variations of chemical indicators contents: melatonin contents of (a) males and (b) females, and cortisol contents of (c) males and (d) females, following the 7-day's schedule adaptation.

circadian rhythm regulation compared to those using the dorm lamp. Lamp 1 and Lamp 3 were special as they caused abnormalities regarding melatonin and cortisol variations with the illuminance of 500 lux. In general, the melatonin contents at 22:00 and 23:00 should be the higher than those at 19:00 and 7:00; correspondingly, the cortisol contents at 22:00 and 23:00 ought to be lower. However, participants employing Lamp 1 and Lamp 3 in 500 lux exhibited abnormally low melatonin level and high cortisol level at 22:00 and 23:00. Effects of Lamp 1 were especially significant even with the decreased illuminance to 250 lux for males.

Each experimenting lamp was turned off at 22:30 in the evening, and participants prepared to go to bed prior to 23:00. There should be few differences in melatonin and cortisol variations between 22:00 and 23:00. However, participants using Lamp 1 and Lamp 3 in 500 lux in the 3rd evening presented lower melatonin contents at 22:00 than those at 23:00 (females: $t = 6.625$ and $p = 0$ for Lamp 1, while $t = 4.712$ and $p = 0.002$ for Lamp 3; males: $t = 5.595$ and $p = 0.001$ for Lamp 1, while $t = 6.218$ and $p = p$ for Lamp 3), and higher cortisol contents at 22:00 than those at 23:00 (females: $t = 10.192$ and $p = 0$ for Lamp 1, while $t = 2.979$ and $p = 0.021$ for Lamp 3; males: $t = 11.706$ and $p = 0.001$ for Lamp 1, while $t = 6.856$ and $p = p$ for Lamp 3).

The 3 lamps used in this study presented similar correlated color temperature yet distinct spectrums in the blue and red region. Lamp 1 presented the blue crest at 467 nm, while the blue crest of Lamp 2 was at 453 nm. Lamp 3 exhibited two blue crests at 453 nm and 467 nm respectively. The abnormities of circadian rhythm regulation caused by Lamp 1 and Lamp 3 were likely to be related to the wavelength distribution, as their spectrums presented high blue proportions, which had been reported to cause melatonin suppression.

Melatonin suppression sensitivity was reported to distribute in the 420-600-nm range [39], [40]. Here we calculated the total suppression of each lamp, using the previously reported wavelength-related suppression sensitivity [41]. Each lamp's SPD data was normalized to 100. Single suppression at each wavelength was calculated by the multiplication of suppression sensitivity and normalized SPD, and the total suppression as the 420-600-nm integral was calculated as the sum of the suppression at each wavelength. Finally we obtained the total suppression of the dorm lamp (with the value 29.72), Lamp 1 (with the value 38.87), Lamp 2 (with the value 31.18), and Lamp 3 (with the value 36.04). For comparison between lamps, ANOVA analysis was performed by comparing the suppression effect at each wavelength using SPSS 20.0. The suppression caused by Lamp 1 was significantly higher than that caused by the dorm lamp ($t = 3.127$, $p = 0.002$) and Lamp 2 ($t = 2.979$, $p = 0.003$). Lamp 3 presented higher suppression compared with the dorm lamp ($t = 5.134$, $p = 0$) and Lamp 2 ($t = 5.371$, $p = 0$). Melatonin suppression caused by the dorm lamp was slightly lower than that caused by Lamp 2 ($t = -2.666$, $p = 0.008$). Lamp 1 and Lamp 3 presented similar effects regarding melatonin suppression without significant difference ($t = 1.614$, $p = 0.108$).

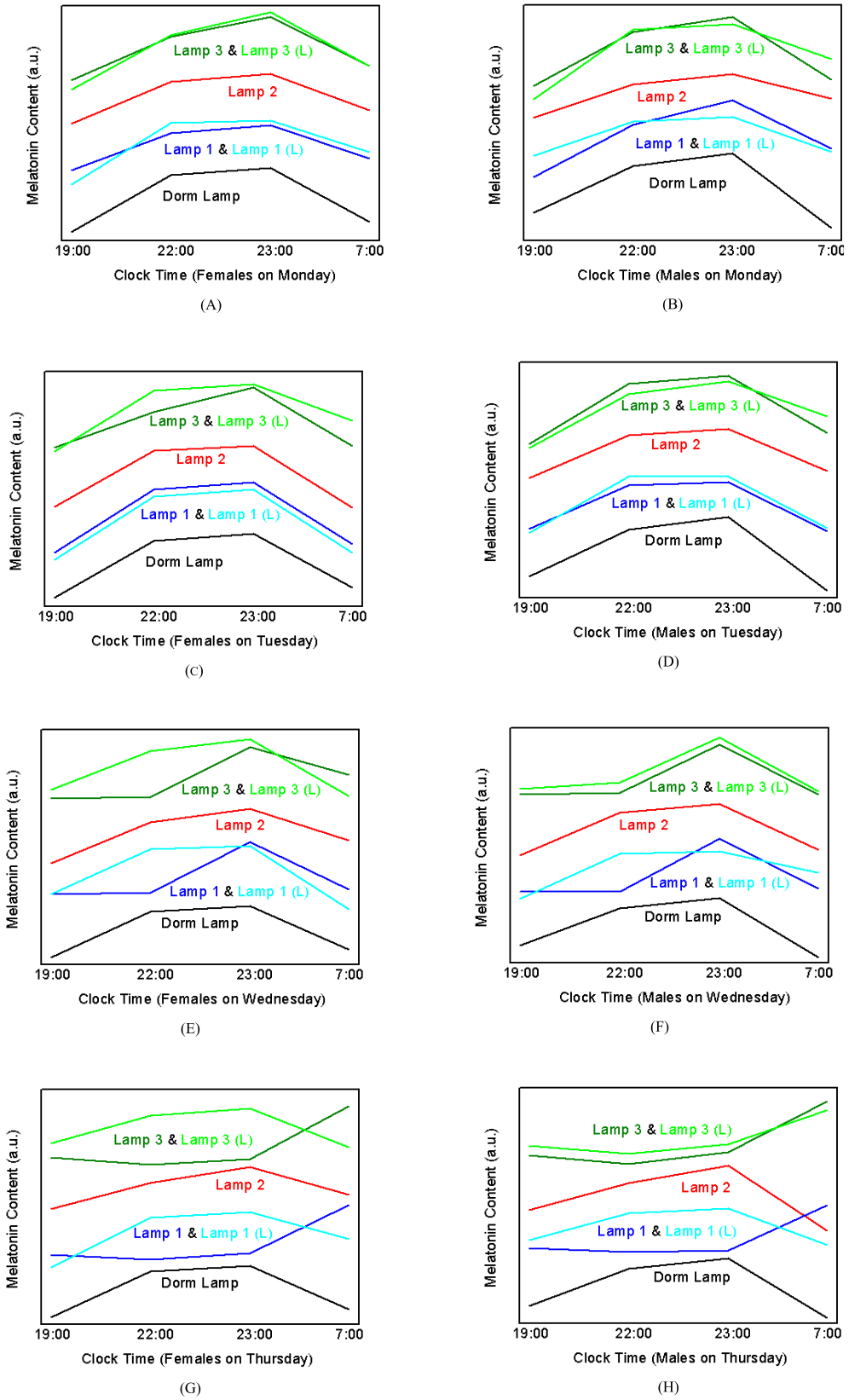

FIGURE 3. Melatonin content variations of females (A, C, E, G) and males (B, D, F, H) using the 4 lamps. Lamp 1 (L) and Lamp 3 (L) represents Lamp 1 and Lamp 3 with low intensity (corresponding to the desk illuminance of 250 lux).

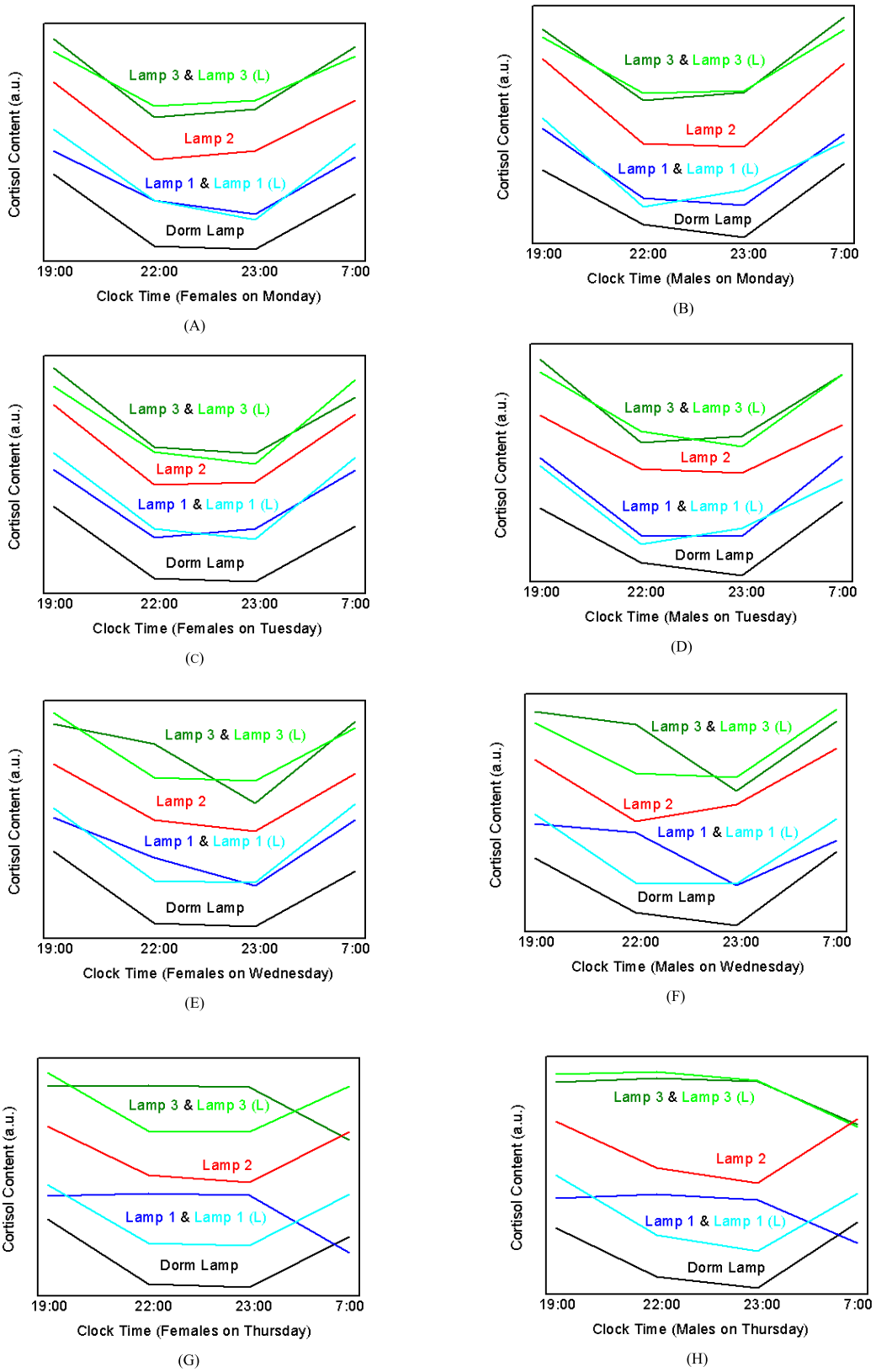

FIGURE 4. Cortisol content variations of females (A, C, E, G) and males (B, D, F, H) using the 4 lamps. Lamp 1 (L) and Lamp 3 (L) represents Lamp 1 and Lamp 3 with low intensity (corresponding to the desk illuminance of 250 lux).

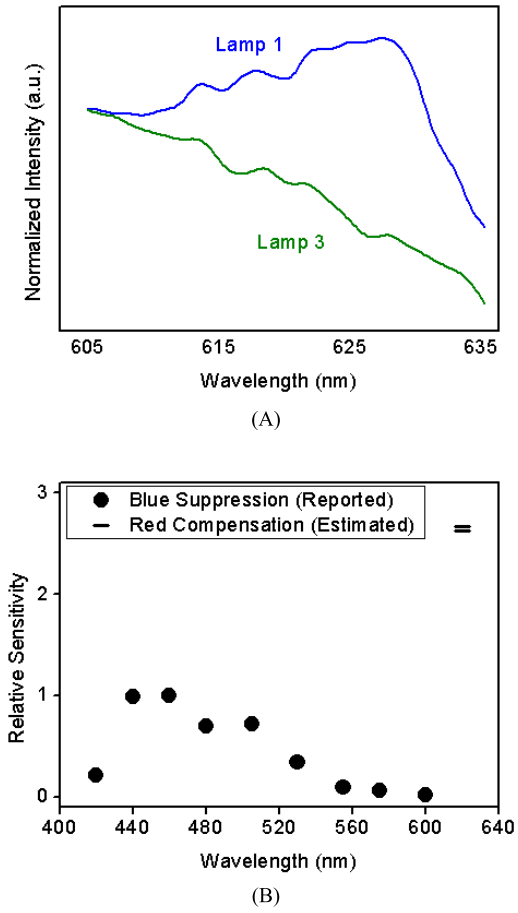

FIGURE 5. (A) Spectrums of Lamp 1 and Lamp 3 in the range of 606-635 nm; (B) Estimated range of M_{620} (solid spheres represent the blue suppression sensitivity reported in previous literatures [39]–[41], while hollow bars represent the range of M_{620}).

Differences still existed in the spectrum characterization between Lamp 1 and Lamp 3, as the blue crest of Lamp 1 was at 467 nm while the blue crest of Lamp 3 was separated at 453 nm and 467 nm respectively. Correspondingly, Lamp 1 had more significant effects on melatonin suppression compared with Lamp 3. Males presented abnormal melatonin and cortisol variations when they used Lamp 1 and Lamp 3 in 250 lux, while the abnormalities disappeared when they used Lamp 3 in 250 lux. Both females and males using Lamp 1 and Lamp 3 began to present abnormalities in the 3rd evening, whereas there seemed to be gender difference regarding sensitivities to the non-visual effects, although we could hardly exclude the possibility of the lacking in dose accumulation that resulted in the insensitive responses of females. Distinctions in effects on circadian rhythm regulation in different illuminances implied the existence of the threshold dose that triggered non-visual effects.

Our results showed that Lamp 3 presented higher suppression even with the illuminance that was reduced by half, inferring that the final suppression was affected by the effects of both blue suppression and red compensation. According to previous researches, the equations are likely to be as follows [39]–[41]:

$$E = S - C \tag{1}$$

$$S = k_1 \int I_\lambda \cdot N_\lambda d\lambda \tag{2}$$

$$C = k_2 \int I_\lambda \cdot M_\lambda d\lambda \tag{3}$$

In the equations above, E represents the final suppression effects, with S representing blue suppression, and C representing red compensation; I_λ represents the wavelength-dependent intensity which is normalized to constant, with N_λ representing the wavelength-dependent sensitivity of suppression, and M_λ representing the wavelength-dependent sensitivity of compensation; k1 and k2 are constants. The value of M_λ was unknown due to the lack of sufficient data, whereas the wavelength in the range of 606-636 nm seemed to be significant due to the spectral difference between Lamp 1 and Lamp 3 (Fig.5). For simplicity, the function M_λ within the range of 606-635 nm was treated as M_{620}, and the constants k1 and k2 were set 1. As the suppression effect of Lamp 3 was higher than that of Lamp 1, M_{620} was estimated as follows according to Eq.1-Eq.3:

$$36.04 - 12.67 \cdot M_{620} > 38.87 - 14.58 \cdot M_{620} \tag{4}$$

As the suppression effect of Lamp 1 was higher than that of Lamp 2, we have:

$$38.87 - 14.58 \cdot M_{620} > 0 > 31.18 - 11.84 \cdot M_{620} \tag{5}$$

The value range of M_{620} was estimated as $2.63 < M_{620} < 2.67$. Further researches were needed to narrow this value range and expand the data range of M_λ.

For participants using Lamp 1 and Lamp 3, melatonin suppression presented phase shifts as the content peak and content trough was delayed in the 4th evening. The phase shift was comprehensible as participants suffering from the lack of sleep were more likely to be sleepy. In the first 2 evenings, Lamp 3 caused normal circadian rhythm regulation due to the cumulative amount of lighting. From the 3rd evening, abnormalities appeared as the lighting effects got enough accumulation. Lamp 1 presented more significant melatonin suppression due to its high intensity in the 460-480 nm range, whereas the suppression effects decayed with sleeping as the ambient light was sheltered by closing eyelids. At 22:30 melatonin suppression was weakened with the experimenting lamp turned off, and melatonin emission was likely to increase without continuous accumulation of lighting in the 3rd evening.

In this study, none of the participants suffered from depression or any mental disease. Cortisol abnormities caused by Lamp 1 and Lamp 3 were likely to be related to the affected emotion originating from interfered sleep, as several participants expressed the perception of drowsiness but insomnia. For participants using Lamp 1 and Lamp 3 in 500 lux, melatonin suppression started at or before 22:00, inferring that

cortisol reduction in participants using Lamp 3 was likely to be caused following melatonin suppression.

IV. CONCLUSION

In the present study, we analyzed the effects of 3 experimenting lamps on circadian rhythm regulations of 17 participants. The 3 lamps were characterized by similar correlated color temperature of 5000K yet distinct spectrums: Lamp 1 with 467-nm blue crest, Lamp 2 with 453-nm blue crest, and Lamp 3 with double blue crests at 453 nm and 467 nm. Following the lighting of Lamp 1 and Lamp 3, significant abnormalities on circadian rhythm regulation appeared on participants with the illuminance of 500 lux, while in 250 lux abnormalities disappeared for females yet still existed for males. Lamp 1 and Lamp 3 were special due to the narrow blue crest around 460 nm, which had been reported to cause melatonin suppression, while difference existed between Lamp 1 and Lamp 3 as the red proportions presented distinct in the 606-635-nm range. We suggest that lighting source designers should increase the 606-635-nm intensity for bed room luminaires to decrease melatonin suppression effects.

Our findings are concluded as follows: (1) Red light in the range of 606-635 nm is likely to compensate for the melatonin suppression caused by the high-blue-intensity spectrum, and the relative compensation sensitivity was estimated in the range of 2.63-2.67; (2) Light-induced abnormity in circadian rhythm regulation requires enough dose, as there seems to be a dose threshold to trigger the abnormity; (3) Circadian rhythm reaction presents gender-related difference, as males tend to be more sensitive to incidence stimulus compared with females.

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