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Research Article

The Influence of Colored Light on Heart Rate Variability and Human Discomfort

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Abstract

Purpose: This study examined changes and relationships between perceived discomfort states and heart rate variability (HRV) during exposure to colored lights. **Methods:** Twenty university students, equipped with a digital Holter recorder, were exposed to white, blue and red lights for 10 min, respectively, with a 5-min interval among these stimuli. HRV series were recorded at rest within each lighting period, and time and frequency domain HRV parameters were analyzed. A visual analog scale assessed discomfort states throughout the experiment. **Results:** Significant increases were found during exposure to the red light in both the low-to-high frequency ratio (LF/HF, $p<0.05$) and low frequency (LF, $p<0.05$), accounting for a heightened activity of the sympathetic system. Blue light stimulation did not induce the same effects in frequency components. Both LF and the LF/HF ratio decreased under blue light condition, whereas high frequency (HF) increased, showing more cardiac relaxation. Compared with red light, blue light improved the subjective measures of discomfort scores. **Conclusions:** Red light exposure during daytime enhances autonomic arousal, suggesting alterations in sympathovagal balance. The moderate increase in vagal-related frequency components due to increased parasympathetic activity is associated with blue light exposure, which may be less stressful than the red one. Perceived discomfort levels correlated with HRV. Although the influence of light on psychological and physiological processes is not fully clarified, the results suggest significant associations between exposure to different colored lights, physiological reactivity and mood, which could be used in the design of lighting interventions both in real-life conditions (e.g., workplace) and in clinical applications.

Keywords: *autonomic nervous system, heart rate variability, light exposure, discomfort state, mood*

Introduction

In the past, effects of light stimulation and light therapy on autonomic activities have been intensively explored [1]. It has been shown that different light intensities can change various physiological measures such as melatonin suppression, alertness, thermoregulation, heart rate (HR), and heart rate variability (HRV) [2]. Furthermore, researchers have demonstrated that color itself can affect physiological, psychological, and behavioural responses in humans [3]. It has been reported that exposure to colored illumination can influence the HRV within minutes and that the effects of different colors can be distinguished by HRV analysis [4].

There is some evidence that the colors of an indoor environment might affect the psychological mood of people in that environment. Comparing red and blue rooms, Küller et al. [5] found increased brain arousal level (assessed as percentage of alpha waves) in people who stayed in a red room. The authors concluded that this effect was particularly significant in introvert persons as well as in those already in negative mood. Most recently, it has been observed that

students living in a university residence hall composed by six buildings that differed only for the interior color (violet, blue, green, yellow, orange, and red), reported a preference for blue interior. Blue as interior color was judged to improve studying activity. In addition, a relationship was found between a positive (calm) mood and preference for blue [6].

However, the accurate effect elicited by specific color exposure on individuals is not fully clarified. A number of studies have found that the color red is more arousing and alerting than the color blue, while other studies have not shown any change in the physiological arousal evoked by the two colors [7]. For example, Choi and co-authors [8] found that the high frequency (HF) power (i.e., the parasympathetic activity) decreased after illumination with red light in subjects with symptoms of anxiety and depression, while it was unchanged in the subjects without those symptoms.

This finding implies that studies investigating the effects of colored lights on individuals should consider these psychological states.

One of the most remarkable roles of light is mood regulation [9]. A psychophysiological study reported that 40 percent of thirty patients with major depression showed improvement of mood during bright-light treatment as well as an increase of the HF power after the bright-light sessions [1]. The efficacy of light therapy on seasonal affective disorders (SAD), a syndrome involving depressive episodes that recur in late autumn or winter, when the photoperiod (hours of daylight) is short, has been acknowledged. A pooled analysis of light therapy studies found that 53 percent of 332 SAD patients met criteria for full remission with light therapy, whereas approximately 40 percent of cases with moderate to severe symptoms remitted with light treatment [10]. These results demonstrated that responders to light therapy were characterized mainly by atypical symptoms closely associated with depressed mood [11]. A light therapy meta-analysis suggests that light of short to medium-wavelengths (blue, green, and yellow) seems to be essential for the therapeutic effect of light in SAD patients, whereas long-wavelength (red) light is ineffective [12].

As a non-invasive method of investigating the autonomic nervous system, HRV records the oscillations of the interval between consecutive heart cycles (RR intervals), which is influenced by the sinus node [13]. Therefore, an effect of light on the autonomic nervous system may be evaluated through time and frequency domain analysis of HRV. More precisely, spectral analysis of HRV provides information about the regulation of HRV by the autonomous nervous system and its 2 antagonistic parts, the sympathetic and the parasympathetic system [14]. HRV, such as other activities of the cardiovascular system, has been stated to depend on circadian phase [15]. Part of the visual information detected by the retina is redirected to the hypothalamus by the retino-hypothalamic tract and to the suprachiasmatic nucleus, a brain area associated with the circadian rhythmicity. From the latter, efferent neural pathways extend to the pituitary gland and centers in the pons and the medulla. Thus, information about light incident on the retina is directed to various brain regions regulating the autonomous nervous system and the cardiovascular system, suggesting a consistent effect of light on HRV [4].

The aim of the present study was (1) to investigate the effects of daytime illumination exposure to different colored light on HRV parameters in individuals and (2) to evaluate the impact of light on emotional state of the participants (i.e., a positive or negative effect on well-being) by a visual analog scale.

Materials and Methods

Experimental design

A total of 20 university students were enrolled in this experimental study after having signed informed consent. All research procedures were approved by the Ethics Committee of the University of Trieste (approval number 80) and were in compliance with the Declaration of Helsinki. The study was carried out in an engineering laboratory – inside a full-scale mock-up of a cruise ship cabin – sitting in the University of Trieste, north-eastern of Italy. In a baseline interview, participants gave information about their health status, medication use, presence of disease, and smoking

history. HRV measurement was performed using a digital Holter recorder. After application of the electrocardiogram (ECG) electrodes to the chest, the tested individuals were required to sit on a comfortable chair at rest in front of a mirror, in the cabin, keeping their eyes open throughout the experiment. The study used a repeated-measures design with 3 different light conditions and took place in April during the day, between 9:00 and 15:00, to minimize the confounding effects of the circadian rhythm. Each person who took part in the experiment, consisting of varying illumination conditions (blue, red, and white light), completed the investigation. The subjects were exposed to colored illumination for 10 min in randomized order. A white light period (baseline condition) of another 10 min preceded or followed the blue and red light conditions. Between the 3 different stimulation modalities there was a resting period of 5 min (i.e., HRV should return to baseline values in this time). Figure 1 summarizes this experimental protocol. After the exposure to the red, blue, and white lights, the participants reported their discomfort levels on a visual scale, adopted from the pain scale of Ellermeier [16], ranging from 0 (positive, comfortable, and relaxed) to 50 (negative, uncomfortable, and anxious), for each light condition.

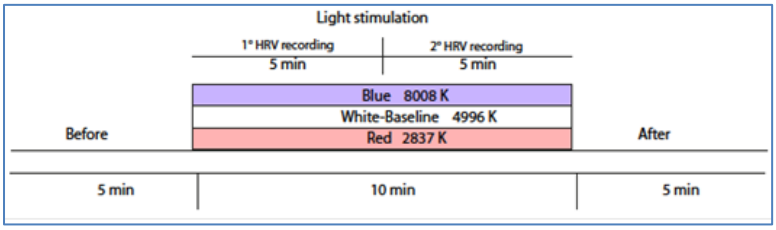

Figure 1: Experimental protocol of lighting conditions and HRV measurements.

Lighting

Ambient light condition has been reproduced through a Philips Hue wireless lighting system. It includes four 10-W Dimmable LED bulbs, a dimmer switch and a base (bridge) that allows remote control (Philips Hue, v. 3.20.1, 7162). Tanks to a dedicated application, it was possible to vary the hue, saturation, and intensity of each bulb at a distance and in real time in order to obtain the desired combination of hue, saturation, and lightness (HSV). The lamps were installed in the cabin in a position from which no direct light reached the participant's eye and was not reflected from the mirror to the subject's eye. The values of HSV were: (1) cool white (Baseline): H0 S0 V100; (2) red/warm low saturation: H0 S30 V100; (3) blue/cold low saturation: H257 S30 V100. From the above values, we extracted the CIE chromaticity coordinates (CIE x and CIE y) assuming a standard white illuminant daylight 50 (cool white-Baseline: 0.3457, 0.3585; red/warm low saturation: 0.4195, 0.3517; blue/cold low saturation: 0.3052, 0.2721). These values were used in order to calculate, according to the McCamy's approximation [17], the correlated color temperature for the cool white (4996 K), the low saturated red/warm (2837 K) and the low saturated blue/cold (8008 K) light. The average luminance reaching the eye of the observer was determined by a Konika Minolta LS-100 photometer. To achieve that, three Munsell color cards corresponding to Munsell values of 8.5, 5.5, 2.5 and the photometer were positioned above a pedestal in correspondence with the point of view of participant. The photometer measured the light quantity reflected from the mirror arriving at each Munsell color card. The average ambient luminance values corresponded to the mean of 3 different measurements recorded for each Munsell color card with the corresponding light condition and resulted in the following values: (1) cool white (Baseline) 6.25 cd/m^2; (2) red/warm low saturation 4.33 cd/m^2; (3) blue/cold low saturation 4.48 cd/m^2. Thus, both light colors had the same saturation (red 30, and blue 30; i.e., the colorfulness, that is how pure a color is) and almost the same dose of luminance.

HRV analysis

ECG monitoring was carried out using a digital Holter recorder (Cardioline clickholter). Beat-to-beat RR-intervals were measured during time periods of 5 min, and on spectral analysis basis HRV was determined. The software

matching the device enabled an export of HRV data into text files that could be analyzed by a computer program which determined time and frequency domain parameters of HRV. For the time domain, we evaluated the following: standard deviation of normal to normal intervals (SDNN) (an estimate of overall HRV), square root of the mean normal to normal interval (RMSSD) (an estimate of short-term components of HRV) and percentage of adjacent pairs of normal to normal intervals differing by more than 50 milliseconds in the recording (pNN50) (high values reflect increased parasympathetic activity). In the frequency domain, low frequency (LF) power (0.04-0.15 Hertz, normalized units) and HF power (0.15-0.40 Hertz, normalized units) as well as their ratio (LF/HF) were analyzed according to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology recommendations [13]. Briefly, high HF power and low LF/HF ratio reflect strong parasympathetic activity, while an increase in LF power is considered a marker of increasing autonomic influence (sympathetic and parasympathetic). Five-min averages of time domain HRV parameters were determined for every 5-min interval with at least 200 beats recorded, and 5-min averages of frequency domain parameters were determined for intervals with at least 300 beats recorded.

Statistical analysis

To perform linear mixed effects (*lme*) analysis of the relationship between the exposure to different light conditions and physiological as well as explicit discomfort measures, we applied R (R Core Team, 2018) and *lme*4 [18].

Individual ratings of estimated discomfort were expressed and analysed as relative scores taking the ratio between the value reported by the subjects on the visual analog scale and the maximum value of the scale (i.e., 50).

The *lme* models included HRV parameters and subjective discomfort measures as dependent variables, light hue as fixed effect (i.e., red vs blue), and participants and the order of HRV recordings (two recordings during each 10 min light exposure) as random intercepts.

We used type 3-like two tailed *p*-values for significance estimates of *lme*'s fixed effects and parameters adjusting for the *F*-tests the denominator degrees-of freedom with the Satterthwaite's approximation [19] based on Statistical Analysis System proc mixed theory. As reliable measures of the predictive power and the goodness of fit for *lme* models, we selected the concordance correlation coefficient r_c, which provides a measure of the degree of agreement between observed and predicted values in the range from -1 to +1 [20,21], and the coefficient of determination r^2. *Post-hoc* tests were performed on *lme* estimated coefficients with paired two sample *t*-tests with unequal variance.

To assess whether the explicit well-being state of participants could influence the effect of light hue on physiological measures, we added to the *lme* models the estimated discomfort ratings as continuous covariates of the HRV parameters. In order to improve this relationship, we split our sample of participants into two groups (*Low discomfort group vs High discomfort group*) on the basis of the median discomfort score. According to Bates [22], we used the same statistical procedure to obtain two-tailed *p*-values by means of likelihood ratio test based on χ^2 statistics. AIC-index was used as a supporting comparative measure of the goodness of fit. The level of statistical significance was set at $p<0.05$.

Results

Twenty healthy volunteers (14 females, 6 males, mean age 22 years) took part in this study. The participants were of average weight, the mean body mass index (BMI) was 20.31 ± 1.81 kg/m^2. None of the subjects received any medication, none of them had cardiovascular disease, or acute visual problems and/or color blindness. Table 1 presents the features of the participants.

Table 1. Baseline characteristics of the study population (n=20).

Variable	Mean ± SD or %
Age (years)	21.95 ± 2.60
Height (m)	1.69 ± 7.92
Weight (kg)	58.65 ± 8.82
BMI (kg/m²)	20.31 ± 1.81
Smokers (%)	3%
HR (bpm)	70.30 ± 5.67
SAP (mmHg)	110 ± 7.75
DAP (mmHg)	69.50 ± 8.05
BMI: Body Mass Index; HR: Heart Rate; DAP: Diastolic Arterial Pressure; SAP: Systolic Arterial Pressure; SD: Standard Deviation	

The analysis in the time domain HRV parameters did not reveal any considerable differences in the prevailing sympathovagal regulation between the three lighting conditions (Table 2). As shown in Figure 2, SDNN, RMSSD and pNN50 did not change during the exposure to red and blue lights (SDNN: $F_{1,37}$=2.44, p=0.1269; RMSSD: $F_{1,37}$=2.13, p=0.1525; pNN50: $F_{1,38}$=0.20, p=0 .6607). The *post-hoc* analysis demonstrated that pNN50 slightly increased during the exposure to white light compared to blue and red ones (white vs blue, $\chi^2 1$=3.91, p=0.0480, white vs red, $\chi^2 1$=3.37, p=0.0663).

Table 2. Average values and their standard error of HRV parameters between the 3 lighting conditions.

HRV Variable	Mean ± standard error		
	Blue light	Red light	White light
SDNN (ms)	67.10 ± 3.64	71.42 ± 3.66	73.20 ± 3.77
RMSSD (ms)	37.97 ± 3.51	40.67 ± 3.92	43.12 ± 3.52
pNN50 (%)	18.72 ± 3.03	18.20 ± 2.96	21.30 ± 2.71
Log LF/HF ratio	-0.09 ± 0.05*	0.02 ± 0.05*	-0.07 ± 0.05
LF (nu)	38.39 ± 0.96*	40.80 ± 1.04*	38.91 ± 1.13
HF (nu)	41.60 ± 1.29	40.22 ± 1.32	42.10 ± 1.33
SDNN (ms): Standard deviation of normal to normal intervals; RMSSD (ms): Square root of the mean normal to normal interval; pNN50 (%): Percentage of adjacent pairs of normal to normal intervals differing more than 50 ms; Log LF/HF: logarithm low frequency to high frequency ratio; LF (nu): low frequency; HF (nu): high frequency. *p<0.05.			

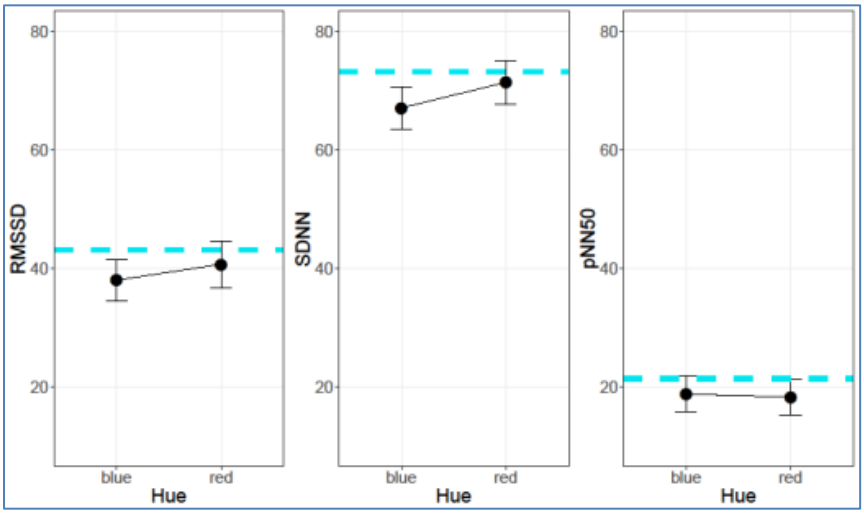

Figure 2: Differences in the time domain HRV parameters during the 3 lighting conditions. RMSSD (ms): Square root of the mean normal to normal interval; SDNN (ms): Standard deviation of normal to normal intervals; pNN50 (%): Percentage of adjacent pairs of normal to normal intervals differing more than 50 ms. The black circles indicate the average values of RMSSD, SDNN, and pNN50 for the blue and red light conditions. The cyan dotted lines represent the average values of the three indices for the white light baseline condition. Error bars represent ± 1 SEM.

In the frequency domain, LF and the LF/HF ratio increased in response to red light exposure but decreased during blue and white light conditions (Table 2). LF and the LF/HF ratio changed significantly during red light exposure compared to blue light exposure (LF: $F_{1,39}$=5.59, p=0.0232, r^2=0.74, r_c=0.75, 95% CI [0.67, 0.81]; LF/HF ratio: $F_{1,39}$=6.99, p=0.0118, r^2=0.83, r_c=0.86, 95%CI [0.81, 0.90]). More precisely, the LF/HF ratio increased of about 0.11 ± 0.04 ($t_{38.57}$=2.64, p=0.0118) and LF rose of about 2.43 ± 1.03 ($t_{38.64}$=2.36, p=0.0232) for red compared to blue light, accounting for an increased level of sympathetic activity. HF was higher during exposure to blue light than red light ($F_{1,38}$=3.41, p=0.0725, r^2=0.94, r_c=0.96, 95% CI [0.94, 0.97]), indicating an enhance in parasympathetic modulation (Figure 3). The *post-hoc* analysis revealed significant changes in the LF/HF ratio and LF for blue compared to red light (LF/HF ratio: $\chi_2 1$=4.87, p=0.0272; LF: $\chi^2 1$=5.42, p=0.0199) and slight differences in the LF/HF ratio and HF for red compared to white light (LF/HF ratio: $\chi^2 1$=3.38, p=0.0661; HF: $\chi^2 1$=4.19, p=0.0406).

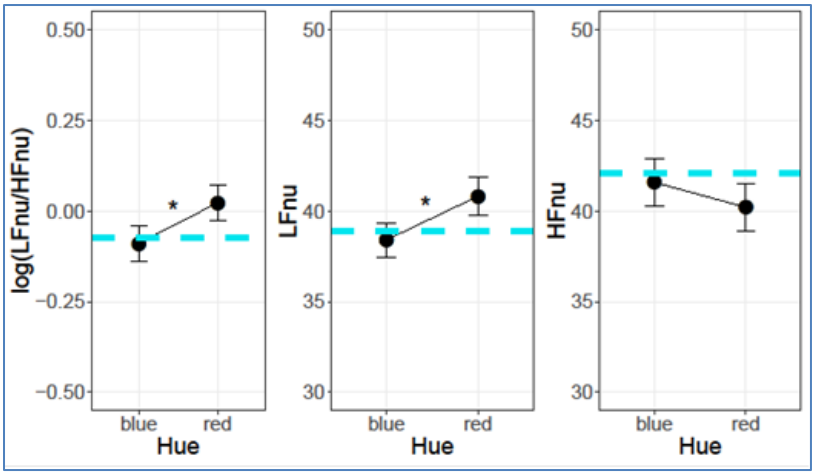

Figure 3: Changes in the frequency domain HRV parameters during the 3 lighting conditions. Log LF/HF: Logarithm low frequency to high frequency ratio; LF (nu): Low frequency; HF (nu): High frequency. The black circles indicate the average values of the LF/HF ratio, LF, and HF for the blue and red light conditions. The cyan dotted lines represent the average values of the three indices for the white light baseline condition. Error bars represent ± 1 SEM. *p<0.05.

The analysis of estimated discomfort ratings did not show a significant effect of estimated discomfort on light hue ($F_{1,39}$=0.05, p=0.8162). However, we found that the explicit estimates of discomfort have influenced the relationship between frequency domain HRV parameters and light hue. The concurrent increases in LF and the LF/HF ratio and the decrease in HF for red compared to blue light were evident only for those participants who perceived themselves in a low discomfort state. Namely, the relaxant or arousing effects of the blue and red lights, respectively, occurred when the participants reported a state of subjective well-being.

These findings were confirmed by the *lme* analysis. Figure 4 shows the trend of changes in HRV as a function of light hue for the *Low discomfort group* (10 subjects, mean age 21.6 years, individual average discomfort rating lower than the median discomfort rating) and the *High discomfort group* (10 subjects, mean age 22.3 years, individual average discomfort rating higher than the median discomfort rating) (see Methods).

In order to improve the 2-way interaction between discomfort ratings and light hue, two further *lme* analyses were performed. The analyses (Figure 4) showed that: (1) the positive relationship between the LF/HF ratio and light hue was reliable for the *Low discomfort group* ($F_{1,19}$=8.76, p=0.0080), but not for the *High discomfort group* ($F_{1,19}$=0.50, p=0.4868); (2) the positive relationship between LF and light hue was reliable for the *Low discomfort group* ($F_{1,19}$=6.58, p=0.0188), but not for the *High discomfort group* ($F_{1,19}$=0.43, p=0.5198); (3) the negative relationship between HF and light hue was almost significant for the *Low discomfort group* ($F_{1,19}$=4.14, p=0.0565), but not significant for the *High discomfort group* ($F_{1,19}$=0.49, p=0.4900).

Therefore, when the comfort state was judged as extremely positive, the LF/HF ratio and LF increase of about 0.19 ± 0.06 (t19=2.96, p=0.0080), and of about 4.13 ± 1.61 (t19=2.57, p=0.0188), respectively, for red compared to blue light, while HF decreased almost significantly of about 1.80 ± 0.88 (t18=-2.04, p=0.0565) for red compared to blue light.

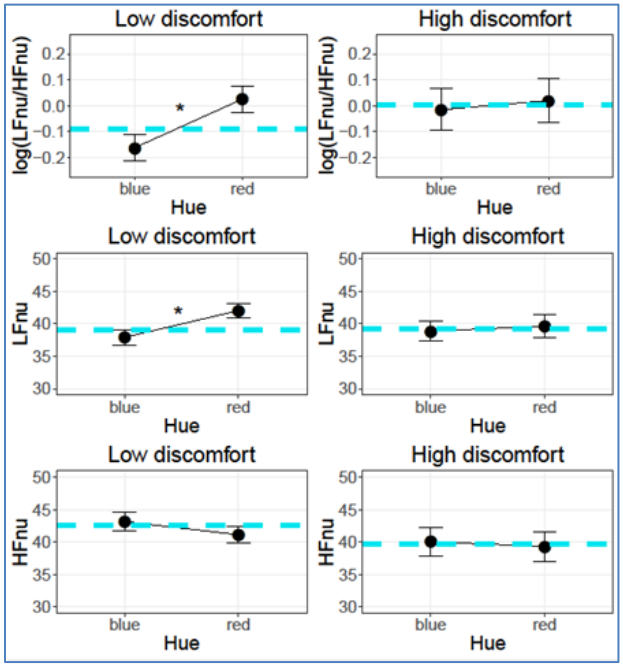

Figure 4. Differences in the frequency domain HRV parameters during the red and blue light exposure for the two groups of participants obtained performing the median split of the discomfort ratings. Log LF/HF: Logarithm low frequency to high frequency ratio; LF (nu): Low frequency; HF (nu): High frequency. The black circles indicate the average values of the LF/HF ratio, LF, and HF for the blue and red light conditions. The cyan dotted lines represent the average values of the three indices for the white light baseline condition. Error bars represent ± 1 SEM. *p<0.05.

Discussion

Traditionally, the effects of light have been investigated at night because melatonin suppression (a marker of the circadian clock) and circadian shifts, which were the first non visual effects to be studied, are the most responsive to light at night. Our results are consistent with the evidence that low intensity blue lighting can enhance positive mood and improve subjective well-being during the daytime, when melatonin levels are low. We found that exposure to low saturation blue light, when compared with exposure to red light of the same saturation, had a calming effect (i.e., increased HF power and decreased LF power and the LF/HF ratio). The increase in HF power suggested an increased parasympathetic activity, whereas the concurrent decrease in both LF power and the LF/HF ratio was consistent with a significant decrease in sympathetic tone. The results also demonstrated that blue light has the ability to improve self-reported measures of well-being state.

Different effects of colored light have been reported in several previous studies. Blue light exposure at night, as compared to green light exposure, induced a greater effect on many physiological parameters, such as melatonin suppression, alertness, body temperature, heart rate, performance and electroencephalographic dynamics [2,23]. Functional magnetic resonance imaging studies have also demonstrated that exposure to blue light during the day, when compared with green or violet light, enhanced responses to a memory task in several cortical, thalamic, and brainstem areas [24]. Furthermore, the exposure to blue-enriched white light in an occupational setting has been shown to improve subjective alertness, performance, mood, and eye strain [25]. Research has discovered that the mechanism through which light produces its non visual effects involves melanopsin-expressing ganglion cells. These effects are

thought to be mediated, at least in part, by projections from the melanopsin-expressing ganglion cells of the retina to the hypothalamus, brain stem, thalamus, and other brain regions that play a role in the regulation of sleepiness, attention, and working memory [26].

Melanopsin, a photopigment expressed in intrinsically photosensitive retinal ganglion cells of the human eye, is specifically sensitive to wavelengths of approximately 480 nm [27]. For this reason, the non visual responses of light are greater when the wavelengths are shorter (blue light) than when the light is geared towards vision. It should be noted that recent studies have shown that long-wavelength light (red, peak close 630 nm) can improve objective and subjective parameters of alertness [28,29]. These results suggest that long-wavelength cones mediate red light's alerting response, given that the photosensitive retinal ganglion cells are not sensitive to long-wavelength light [30].

In the current study, we found that LF power and the LF/HF ratio were higher during exposure to red light compared with blue and white lights. Changes in the LF/HF ratio may provide information about the balance between sympathetic and parasympathetic modulations. Therefore, an increase in both LF power and the LF/HF ratio is more consistent with increases in sympathetic activity than decreases in parasympathetic tone.

The importance of HRV as an index of emotional state regulation has been investigated by Elliot and co-authors [31]. The authors reported that exposure to red light (versus a control color) decreased the parasympathetic activity, as indicated by the reduction of HF index, and induced a worse cognitive performance. Others found a decrease in the LF/HF ratio after illumination with "cold" colors [32]. We can confirm these results. However, it is known that HRV is affected by changes in the individual psychological state [8].

Subjective assessments were made in our sample using a visual analog scale which demonstrated the positive effect of blue light. Indeed, we found that exposure to blue light could substantially change the discomfort rate in the sense of positive effect of well-being. In contrast, exposure to red light evoked more frequently anxiety, tiredness, or other unpleasant feelings.

HRV reflects the activity of the autonomic nervous system. According to a well-known model [13], it is stated that HF power corresponds to the parasympathetic nervous activity, whereas LF power mainly reflects the activity of the sympathetic nervous system. Another interpretation [33] considers LF as an indicator of both the sympathetic and parasympathetic nervous system activities. In both models, the heart response to increased parasympathetic nervous activity is faster than the response to increased sympathetic nervous activity. After exposure to different lighting conditions, our participants reported their subjective evaluations. They judged the red light more arousing and less pleasant than the blue light. We compared the subjective measures to the significant changes of LF power and the LF/HF ratio (i.e., the increase for red and the decrease for blue) and the contrary behavior of HF power. The responses observed in our study are consistent with the second model and may be explained by an increased sympathetic and parasympathetic activities stimulation induced by red and blue lights, respectively. If we consider the first model with its division into rigid spectral bands (LF and HF) reflecting the physiological mechanisms of these components [4], we observe that HF power shows no evident change to different lighting conditions, while LF power and the LF/HF ratio reveal the most marked changes, related to increased sympathetic activity. On the contrary, we suppose, according to the second model, that the spectral activities of the two parts of the autonomous nervous system overlap in LF power [4].

Our study underlines that HRV analysis can be a reliable tool to estimate the influence of different light colors on physiological responses during a short time exposure. Time domain parameters remained unchanged during the different lighting conditions. Such results are coherent with the literature [4] and suggest that these parameters reflect the general state of the cardiovascular system and its capacity to adapt to external demands.

An important variable that we investigated was the low saturation of different light colors. We have shown, through the use of a robust study design (e.g., calculating 5-min averages of light exposure and ECG parameters made an

adequate number of repeated within-subject data available), that exposure to low saturation light colors (red and blue) can have different effects on the HRV parameters and discomfort rate. These results do not imply that light colors with different saturation (i.e., higher) could induce the same physiological effect which we found in this study.

Previous studies have investigated the relationship between colored light and several arousal-related physiological measures, suggesting that not hue but rather variations in saturation caused the arousing effect of red light [34]. Based on these considerations, our experiments were performed under controlled conditions, measuring not only luminance (intensity), but also saturation of colored lights. Taken together, the participants in our study experienced red light as less pleasant and more arousing than blue light with the nearly same intensity and the same saturation. As a matter of fact, analysis of HRV in our sample, showed increased levels of autonomic activity during exposure to red light, which were correlated with subjective feelings of discomfort. On the contrary, daytime exposure to blue light enhanced the participant's relaxation and thereby improved subjective well-being. Some studies on light therapy and color may support our findings [12]. Recently, a field study demonstrated that low-level "bluish-white" lighting (which emits shorter wavelength content) improved sleep quality and behavioral symptoms, such as agitation and depression, in patients with Alzheimer's disease and related dementia [35].

In line with previous research [25], our data suggest a relationship between arousal (sympathetic or vagal), mood (stressed or relaxed, anxiety or calm) and exposure to different colored lights (red or blue light). Here, we show improvements in subjective well-being and arousal (i.e., increased relaxation parameters in the frequency domain) as a result of short-time blue light exposure. These effects could have substantial benefits on worker safety in a variety of occupational environments (e.g., office or healthcare settings) as many activities are performed indoors with artificial lighting conditions and/or at night time.

For example, the alerting response to blue light has been consistently reported in literature and thus the ability of short wavelength (blue) light to enhance alertness and cognitive performance has been assessed as a non-pharmacological countermeasure for drowsiness in a range of occupational settings, particularly in shift or night workers [23]. However, a field trial has shown that exposure to blue-enriched white light during daytime work hours in an office setting had a positive effect on subsequent sleep quality and duration, which contrasts with the negative effects on sleep that have been reported with other alertness-enhancing interventions, such as caffeine administration [25]. According to the authors [25], this would indicate that the effects of blue-enriched light on sleep may be a consequence of improved daytime activity. Alternatively, it was hypothesized that daytime exposure to blue-enriched light would increase the participants' nocturnal melatonin secretion, improving sleep quality, which in turn may have contributed to enhanced daytime alertness, performance and mood in the workplace. Blue-enriched light exposure also demonstrated positive effects on visual functions, and it was associated with a decrease in eye strain, discomfort, fatigue and blurred vision when compared to white light exposure. It has been suggested that this improved visual comfort may be related to the impact of the melanopsin system on the visual system [25].

The present study addressed the question of HRV changes under environmental stimuli (i.e., different lighting conditions) indicating a high sympathetic arousal -correlated with subjective feelings of discomfort- in the participants during exposure to the red light, which was more stressful compared to blue and white light exposure. In many work environments, the simultaneous presence of different environmental factors can have cumulative effects on cognitive and physiological functions that might led to a decrease in comfort and working efficiency [36]. On the contrary, a proper ambient lighting of a workplace improves visual performance enabling people to perform tasks accurately, quickly and without unnecessary effort, and this may enhance employees' satisfaction and safety in their work environment [36].

The link between blue light exposure, arousal and subjective well-being, reported in our laboratory study, can have practical implications on environmental ergonomics. The latter (including lighting condition) should have a more important role to play in the design of the workplace in order to improve visual comfort, performance, mood and safety, as suggested in previous research [37].

A contemporary view of environmental ergonomics includes not only the physical environment such as thermal conditions, ventilation and lighting, but also the social, psychological, cultural, and organizational environments of systems. Among the environmental factors, inadequate lighting condition can contribute to a worker discomfort and also affect human activity and visual performance [38]. Although it is well known that the physical environment has a significant impact on human health and safety, hospital design is, for example, one of the factors that can potentially affect staff performance [39]. It has been found that poor lighting is associated with healthcare worker falls in hospitals [40] as well as with medication errors [41].

A field study examined the effect of lighting conditions on employee satisfaction, job performance, safety and health in a hospital setting using both questionnaire and physical illuminance measurements. The results demonstrated that about two-thirds of the participants reported that at least one of the characteristics evaluated in the study (light level, type of light sources, light colour and use of daylight) was inappropriate, showing that light level was strongly correlated with employees' satisfaction, job performance and eye tiredness. These findings evidence that physical measurements should be supported by subjective assessments in order to consider various aspects of lighting conditions in working environment, improving healthcare facilities [37]. This is the approach used in the experiment that we reported in our research. It is worth noting then, that light color was also correlated with employees' satisfaction [37], consistently with our laboratory data.

Concerning the health and safety problems, health care personnel reported that low light levels in the work environment caused eye tiredness to them and the need to change their body posture for a better visibility of the objects or working items [37]. From the ergonomic point of view, this finding is important because the inadequate postures and uncomfortable positions, during performing activities, are considered significant risk factors in the occurrence of musculoskeletal disorders among health care workers.

This study design (i.e., exposure to different light colors in the same persons during a relatively short time and during identical steady-state laboratory conditions) minimized intra- and inter individual subject variability. Moreover, the period (i.e., spring month, April) and location (i.e., Trieste, north-eastern of Italy) of the trial ensured that the contribution of artificial light to the total light exposure was substantial.

At this point several limitations must be considered. Firstly, the low number of subjects included in the study. However, there were significant changes in HRV parameters which made a common interpretation meaningful. Secondly, the study population consisted mainly of young healthy females. Thus, our observations may not be generalized to other populations (e.g., elderly people). Thirdly, since we did not measure melatonin secretion, we were unable to determine whether the light exposures affect the entrainment to environmental light-dark cycles or not. Finally, a possible response bias is usually inherent in self-report information like the discomfort level score in our study.

By and large, lighting researchers have been tied to thinking about a particular building- that is, a single place where one needs to see for tasks and perceive environment instantaneously. Today, because people have active lives that change their 24-hour pattern of light and dark, they do not have a single lighting entity that is responsible for total 24-hour light exposure patterns. It is well-known that a regular 24-hour light-dark pattern minimizes circadian phase disruption, which become an issue when people travel across multiple time zones or spend most of their days in dim daytime interiors [42]. Indeed, we can hypothesize that people like airline pilots, cruise ship staff, and shift worker are unlikely to have an ideal lighted environment. Nevertheless, it is important that scientific research continues to

investigate about how light can be used to improve health quality and positive mood, as well as the ways to minimize circadian phase disruption, which in turn minimizes negative health and discomfort outcomes.

In sum, the study suggests changes in HRV parameters in association with individual day-time exposure to different colored lights, possibly mediated by a sympathovagal imbalance. Our data also demonstrated that the objective, measurable effects were associated with subjective impressions of participants. Although the results of this study are consistent with other studies, it is difficult to draw any firm conclusions from it because the different effects of colored light as environmental stimulation of human health are not fully clarified.

From a theoretical point of view, another question that arises is whether these results are influenced by personality trait-specific stress coping in individuals. Methodologically, there is a need to simultaneously evaluate both relaxation and stress states to improve understanding of the person's own capabilities to positive or maladaptive response to stressors, such as environmental stimuli. Actually, individuals' experience levels of stress are likely to include several dimensions such as social variables (e.g., academic or occupational demands) and psychological variables (e.g., emotions, coping strategies, defense mechanisms). The interaction of these multiple stressors may have negative effects on their well-being.

Therefore, further studies about the relationship between light colors stimulation and cardiac autonomic regulation are necessary to expand the potential effect of colored light into therapeutic applications as well as in real-life conditions, like occupational setting.

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