

Influence of Wearing Blue Lenses on Melatonin Production and Performance in Volleyball Players



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ABSTRACT

We analyzed the effects of wearing blue lenses on melatonin level, physical and cognitive performance. Fifteen youth volleyball players (15.0 ± 1.5 yrs) attended the laboratory on 3 occasions (48-h interval): on the 1st visit they were familiarized with the procedures of the study, and on 2nd and 3rd visits they were submitted to the testing protocol wearing transparent (control) or blue lens glasses in a counterbalanced crossover design. The protocol consisted of 10 min in “total darkness,” 30 min of light stimulation (wearing blue or transparent lenses), followed by an attentional test, and an agility T-test (without wearing the glasses). Samples of saliva (to determine melatonin concentration) were obtained pre- and post-exposure (30 min) to artificial light, wearing the lenses. Sleepiness, alertness, attention, mood, and perceived recovery status and performance variables (reaction time and T-test) were assessed after lens exposure. Melatonin levels did not differ within and between groups (blue lenses, pre: 0.79 ± 0.73 and post: 1.19 ± 1.374 pg/dl, $p = 0.252$, effect size (ES) = 0.38; control, pre: 0.97 ± 1.00 and post: 0.67 ± 0.71 pg/dl, $p = 0.305$, ES = -0.35). Nonetheless, melatonin differences were significantly correlated with physical sedation for glasses with blue lenses ($r = -0.526$; $p = 0.04$). No other variables differed ($p > 0.05$) between protocols, including T-test performance ($p = 0.07$; ES = 0.41). Blue lenses do not influence melatonin levels, cognitive/physical performance, and mood status in amateur youth volleyball players.

Introduction

Biological processes such as cardiac, endocrine, and brain processes have standardized rhythms under the environmental stimulation of the presence (or absence) of light [1]. Vision is a means of receiving light stimuli and maintaining circadian synchronism, as one of the most important senses in humans. Light exposure is the main stimuli for synchronizing circadian rhythms, releasing hormonal alterations, and influencing motor and cognitive tasks and even athletic performance [1].

In this context, melatonin plays a role in the regulation of a variety of physiological processes such as the circadian clock in the suprachiasmatic nucleus, vascular response, reproduction, sleep and cognition [2, 3]. Light exposure to different illumination patterns is a feasible and low-cost tool and could help improve depression and anxiety recovery, alertness state, and performance. The relationship between exposure to light and decreased melatonin secretion has been documented [4], and sports scientists showed that lower levels of melatonin were associated with greater speed in reaction time, an important indicator of the cognitive processes in sports [5–10].

Volleyball is a team sport involving intermittent and unpredictable actions, with intense physical demands interspersed with moments of pause [11]. Volleyball players must have a high cognitive and attention flexibility (broad and selective) for excellent performance [12]. However, competitions often take place at night, when most athletes are past their peak performance period of the day, and exposure to light can lead to a decrease in melatonin levels. A lower melatonin level might decrease the natural loss in reaction time that occurs after long periods of wakefulness [13].

The effect of monochromatic blue can be even greater in suppressing melatonin levels [14, 15]. However, there is scarce knowledge about the effects of wearing glasses with blue lenses on improving sports performance and pre-activation of the motor cortex of the central nervous system. Any improvement in alertness, and cognitive and physical performance potentially could benefit sports performance (e. g., volleyball) and have a practical application. Thus, the aim of the present study was to analyze whether wearing glasses with blue lenses under an artificial light pattern would influence the melatonin level, physical and cognitive performance of youth volleyball players. Our hypothesis was that wearing blue lens glasses would promote acute suppression of melatonin production (via modulation of the central nervous system), triggering improvements in alertness, cognitive performance, and agility.

Materials and Methods

Subjects

Fifteen youth volleyball players participated in this study (15.1 ± 1.5 years; 180.9 ± 11.5 cm; 76.6 ± 13.9 kg, body mass index 23.4 ± 3.9 kg/m²). Their usual awakening time was between 6:30 am and 10:40 am. The research project was approved by the local Ethics and Research Committee (n. 68569417.5.0000.5147). The volunteers and their parents signed a free and informed consent form before the beginning of the experiments. The following inclusion criteria were used: 1) male gender, 2) participant in competitive youth volleyball

team, 3) abstained from exercise within 24 hours prior to testing, and 4) having a chronotype between 1 (moderately morning) and 5 (moderately evening). Since our goal was to avoid a predominantly morning or evening sample, we believed that the above chronotype description was an inclusion criterion.

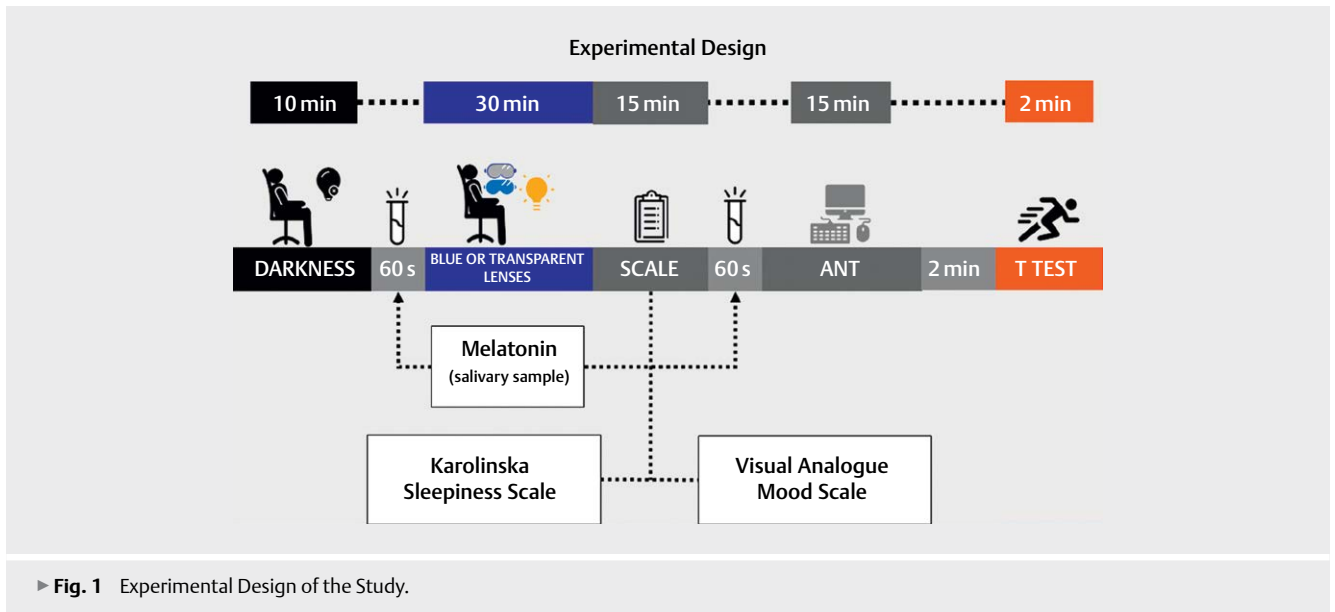
Exclusion criteria were: 1) having a history of injuries that compromised the tests; 2) having used dietary supplements or medications that could affect performance for at least 2 weeks before testing; 3) present health problems that prevented the procedures from being performed; 4) having any visual impairment related to the distinction and visualization of colors; and 5) having changed time zones in the week before the tests.

Experimental design

The players attended the laboratory on 3 occasions with a 48-h interval in between. On the first visit, they were characterized by age, body mass, height, and body mass index (weight/height²), and reported their time of experience in volleyball. The athletes also received the Pittsburgh Sleep Quality Index (PSQI) [16] and the Morningness-Eveningness Questionnaire [17] to be delivered completed on test days. They were then asked to abstain from intense physical activity and the use of alcoholic beverages and/or stimulants 24 hours prior to the tests, and to maintain their usual bedtime and waking time the day before the tests and on test days. Finally, the athletes were familiarized with the procedures of the experiment.

On the other two visits, the glasses (blue or transparent) were randomly selected for the participants, who performed the experimental procedures in a counterbalanced manner. Initially, the athletes were sent to the laboratory where they remained seated with their arms and head supported, avoiding any kind of sudden movement. This position was maintained for a period of 10 minutes in “total darkness” with their eyes totally blindfolded by a mask and with all the lamps out, with 0 lux, verified through the lux meter (Victor 1010A Auto Digital Lux Meter). The period of “total darkness” was followed by the first collection of saliva, which was labeled and stored in an ultra-freezer at -80°C . Soon after, the individuals were submitted to light stimulation. The light stimulus was modified by wearing the blue or transparent lenses (according to a drawing). The use of lenses was carried out only during the period in which the subjects remained in the condition of light stimulation. At the end of this period, each participant was asked, “How do you feel right now?”. The athletes also checked the Karolinska Sleepiness Scale [7, 18, 19], their current state of alertness or drowsiness, and the Visual Analogue Mood Scale [7, 20]. Upon completing these procedures, a salivary melatonin sample was again collected, labeled, and stored in an ultra-freezer. After the second collection of the saliva sample, the athletes were taken to an annex of the physiology laboratory for the Attentional Network Test [21]. Thereafter, they were taken a little further to the yard for the T-test. ► **Fig. 1** shows the experimental design of the study.

All data relating to the attentional network test and the T-test were noted on a form and allocated to a file within the physiology laboratory in conjunction with the Sleep Quality Morningness-Eveningness Questionnaire, Visual Analogue Mood Scale, Karolinska Sleepiness Scale, and subjective recovery scale.



► **Fig. 2** Blue Safety Glasses.

The environment

The ambient lighting was provided by 12 fluorescent lamps of the Philips TLD 32 W/840-NG Super 84 Eco Master tube, each with a luminous flux (lumens - lm) of 2700 lumens, a temperature of 4,100 kelvin, a wavelength of approximately 550 nanometers, a power of 32 watts, with a total size of 121 centimeters. They were positioned in pairs horizontally by means of six TBS050 luminaires (Embed), with a general diffuse lighting characteristic, and a distance of 2.50 meters from the light source to the ground. The average lux of the laboratory was calculated using the formula: Luminous flux (lumens)/illuminated surface area (meters²), calculated as $(2,700 \times 12)/39.4 \approx 822$ fluxes, complying with ICS 91.160.10. The temperature ($22 \pm 1.0^\circ\text{C}$) and relative humidity ($66 \pm 2.0\%$) of the environment were controlled.

Blue monochrome color lenses

The lenses (► **Fig. 2**) are composed of yellow, green, and red spectrum protection lenses (Blue Safety Glasses 492 nm–770 nm) in a blue monochrome color and transparent lenses (without protection). ► **Fig. 2** shows the blue lens goggles used.

Morningness-Eveningness Questionnaire

To identify the chronotype, the Horne and Ostberg's Morningness-Eveningness Questionnaire [17] was used, translated into Portuguese by Benedito-Silva et al. [22]. This is a questionnaire of self-assessment that categorizes a person based on their preference for performing routine activities in the morning or evening. The result is a numerical value that varies between 16 and 86 points, classifying the individual in 5 (five) different types: extreme afternoon (16 to 30 points), moderately afternoon (31 to 41 points), indifferent (42 to 58 points), moderately morning (59 to 69 points) and extreme morning (70 to 86 points).

Sleep Quality Index - PSQI

The PSQI [16] translated into Portuguese [23] was applied to assess the subjects' sleep quality for the last month. The questionnaire consists of 19 self-administered questions and 5 questions answered by a roommate (only used for clinical evaluations). Thus, the 19 questions are grouped into 7 components, distributed on a scale of 0 to 3. These PSQI components are divided into subjective "sleep quality," "sleep latency," "sleep duration," "habitual sleep efficiency," "sleep disorders," "use of sedative medications," and "daytime dysfunction." Therefore, the scores for these components are added to produce a global score, which ranges from 0 to 21, with the higher the score, the worse the quality of sleep. A PSQI greater than five indicates that the individual is experiencing great difficulties in at least 2 components or moderate difficulties in more than 3 components.

Illuminance measurement

Illuminance is defined as the luminous flux, the amount of light that reaches a certain point. The unit of measure of illuminance is expressed in lux and was measured using the Victor 1010 A Auto Digital Lux Meter. According to ICS 91.160.10, to correctly use the lux meter, the evaluator must maintain the device at an illuminance similar to that of the environment for 5 to 10 minutes for stabilization,

perform measurements on the work plane, and maintain a minimum distance of 2 meters from the lux meter cell so that the luminous flux is not influenced by the person taking the measurement. All measurements were performed at the participant's eye level.

Salivary melatonin measurements

All pre-test samples were collected between 18:00h–18:30 h, the period in which the beginning of the melatonin synthesis threshold in low light begins to increase significantly. All samples were collected in a tube (salivary kit collection 1 ml - melatonin), with all individuals seated, with at least 0.5 milliliters of saliva collected. The melatonin analysis was performed using the Automated Enzyme Immunoassay analysis kit (IBL International, Hamburg, Germany). The minimum detectable dosage of melatonin (analytical sensitivity) was determined to be 0.30 pg/ml. Salivary samples were collected following previous recommendations [24, 25].

The participant stimulated the production of saliva and deposited it in the bottle until reaching the equivalent of at least 0.5 ml. Immediately after salivary collection, the vial was identified with the athlete's name and labeled pre or post and blue or transparent lenses. Duly identified, it was deposited in a polystyrene box in an upright position and stored in an ultra-freezer at a temperature of -80°C for further analysis.

Karolinska Sleepiness Scale

In order to check the subject's state of alert and sleepiness, the Karolinska Sleepiness Scale [18] was applied. The scale consists of 9 points, where each item features a characteristic: 1 = very alert, 3 = alert, 5 = neither alert nor sleepy, 7 = sleepy, 9 = very sleepy. After the light exposure, the subjects were asked to visualize the scale to verify their real state at the moment.

Visual Analogue Mood Scale

The Visual Analogue Mood Scale [26], translated to Portuguese [20], consists of 16 items. Each of them was represented by a straight line of 100 millimeters connecting two opposite feelings. Four intuitive factors were combined into these items: anxiety, physical sedation, mental sedation, and other feelings. Before applying the scale, previous training was carried out, featuring oral instructions and practical examples about the scale. Furthermore, it is important that oral instructions emphasize that both ends of the line should be considered the maximum the subject can feel with respect to that item and the center is equivalent to its usual state. Hence, the subject filled each item by crossing the line that links the two opposite characteristics in all sixteen items on the scale.

Attentional Network Test

The Attentional Network Test (ANT) developed by Fan et al. [21] includes a computer test featuring "opposed" tasks and their respective answers verified through the reaction time (RT) in milliseconds and percentage of correct answers. In addition, the ANT requires the subject to determine in a set of five arrows whether the central object is pointing left or right. As a reference, the arrows appear above or below central point in the screen, accompanied or not by arrows indicating opposite (incongruent) or equal (congruent) sides. Thus, ANT's efficiency is assessed by measuring

how the RT is influenced through the warning tips, spatial tips, and congruence of the arrows. Moreover, executive functions such as alertness and guidance are also assessed through the ANT. The session consists of a practical part that lasts for 2 minutes and contains a block of 24 models, presenting the right and wrong answers during the subject's practice. After that, the experimental model is generated, showing a total of 3 blocks of 5 minutes, each block presents 96 models randomly, without any feedback response. Therefore, the ANT test is used to test refined motor skills, with a high demand for precise movement made by small muscle groups, which generally involves high levels of coordination between the eyes and hands [13].

T-test

An adapted version of the T-test [27] was used with its measurements reduced. This version of the test consists of a frontal and posterior move of 5 (five meters), and two opposed lateral moves of 2.5 (two meters and fifty centimeters).

The test site was previously marked with orange paint spray for positioning the "T" shaped cones. Four cones 24 cm high were placed in each marked space and named A, B, C and D. The athlete had to stay just behind cone A and wait for the call to start that was given from the countdown of 3 (three) seconds. The athlete quickly moved forward to cone B, then moved laterally to cone C and laterally to cone D. Finally, the athlete moved laterally to cone B and later to the beginning at cone A. When the subject reached the cones B, C, and D, a squat movement was performed, followed by touching the fingertips to the respective cones. The total test time (in seconds) was recorded by two evaluators using two Samsung Galaxy S6 stopwatches when the athlete reached cone A, signaling the end of the test. Trials with more than 3% difference were not considered.

Subjective Recovery Perception Scale

To verify the athletes' current recovery level, the Subjective Recovery Perception Scale (SRP) [28] was utilized. The SRP has reference values from 0 to 10 in the extremes, meaning "extremely tired" and "very well recovered," respectively.

Statistical analysis

The normality of the data was verified by the Shapiro–Wilk test. For melatonin response, two-way analysis of variance (ANOVA) repeated measurements followed by Bonferroni's post hoc test was conducted to assess the interaction between time and intervention. For the other variables, the Student's paired t-test or Wilcoxon test was performed to verify differences between interventions. In addition, to verify correlation between melatonin differences and cognitive and performance parameters, a Spearman's test was performed. IBM SPSS statistical software (Version 20; IBM Corp., Armonk, NY, USA) was used to perform data analyses. The level of significance adopted was $p < 0.05$. Cohen's d' effect size (ES) were calculated and magnitude was classified as: < 0.2 = trivial, 0.2 – 0.6 = small, 0.6 – 1.2 = moderate, 1.2 – 2.0 = large, and > 2.0 = very large [29].

Results

Using a post-hoc statistical power test with 15 participants, a power of 0.76 was reached [G* Power Software (Dusseldorf, Germany); statistical test = ANOVA: repeated measures, within-between interaction; $\alpha = 0.05$; ES = 0.37; number of groups = 2; and number of measures = 2]. All volleyball players were exposed to the same illuminance level (blue lenses, 352.4 ± 35.9 lux; transparent lenses, 349.2 ± 35.1 lux; $p = 0.834$, ES = 0.09).

There was no time effect [$F(1, 14) = 0.035$, $p = 0.854$] or interaction between time and intervention [$F(1, 14) = 3.576$, $p = 0.08$] for melatonin. In both conditions [(blue lens, pre: 0.79 ± 0.73 to post: 1.19 ± 1.37 (pg/dL), $p = 0.252$, ES = 0.38; colorless lens, pre 0.97 ± 1.00 to post 0.67 ± 0.71 (pg/dL), $p = 0.305$, ES = -0.35], post hoc analysis showed that melatonin did not change within and between groups (► Fig. 3).

There were no changes between blue and transparent lenses on the Karolinska Sleepiness Scale (Blue, 4.2 ± 1.7 KSS; colorless, 4.9 ± 1.2 KSS; $p = 0.148$; ES = -0.48), although a small ES was found, and the Visual Analogue Mood Scale (► Table 1).

Concerning ANT, there was no significant difference in the number of correct answers ($p = 0.308$) between blue and transparent lenses. The same was observed to time reaction ($p = 0.698$) and T-test performance ($p = 0.066$). However, as for T-test performance, a small ES was found. These results are displayed in ► Fig. 4.

A negative significant correlation was observed between melatonin differences and physical sedation for glasses with blue lenses. No other significant correlations were found (► Table 2).

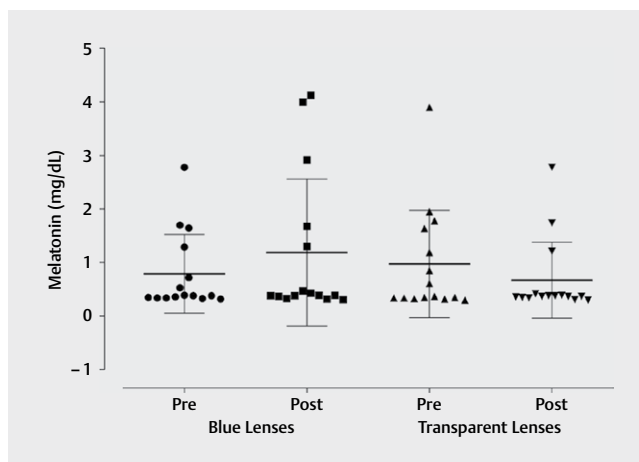
Discussion

Although a previous investigation evaluated the acute effects of wearing colored-lens glasses on exercise performance and testosterone concentration [30], in this study we tested the effects of wearing blue lenses under artificial light conditions in melatonin responses, and physical and cognitive performance. Overall, we found no significant differences when assessing salivary melatonin, alertness, mood, and performance variables (reaction time and T-test).

In contrast with previous investigations [6, 7, 31–33], light exposure in our study did not reduce salivary melatonin level. However, these studies used a longer period than 30 minutes, suggesting that the time of exposure to light may influence the outcome.

Some studies have shown a correlation between subjective alertness and measures of cognitive performance [34, 35]. In light of these results, Zhou et al. [36] proposes that the subjective alertness may not reflect an improvement in cognitive performance and vice versa, and that a reduction in alertness does not always reflect some impairment in cognitive performance tasks. The present study does not present a significant difference in the alertness level, nor did it demonstrate improved cognitive performance from the use of blue lenses, given the lighting condition. However, other studies demonstrated that exposure to light caused changes in both objective and subjective measures of alertness and improvements in cognitive performance [7, 37–39].

An important aspect of our results is that a complex psychomotor test was used to analyze the objective measure of cognition. No relevant changes were observed in the reaction time with the use



► Fig. 3 Melatonin responses before and after exposure of the blue and transparent lenses. Data are expressed as individual values \pm SD.

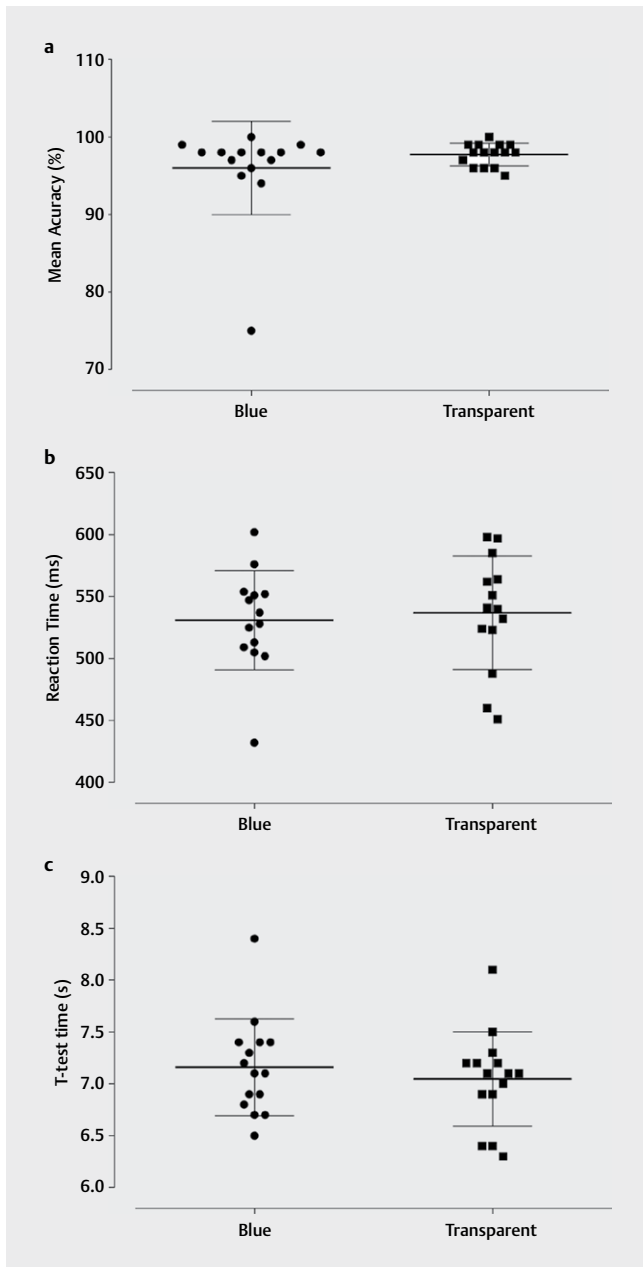
► Table 1 Visual Analogue Mood Scale: results between blue and transparent lenses.

	Blue lens	Transparent lens	P _{value}	ES
Anxiety (mm)	177.0 \pm 30.0	168.0 \pm 19.0	0.589	0.17
Physical sedation (mm)	315.0 \pm 80.0	327.0 \pm 66.0	0.532	0.05
Mental sedation (mm)	107.0 \pm 17.0	110.0 \pm 34.0	0.875	0.20
Other feelings (mm)	165.0 \pm 40.0	177.0 \pm 47.0	0.570	-0.17
Median \pm interquartile range; effect size, ES.				

of lenses with filters and without filters. These results may be due to the non-specificity of the test. The reaction time reflects the nature and duration of the cognitive processes, intervening in successive stages of information processing, between presentation of the stimulus and the response [40]. This proves to be of fundamental importance for volleyball players because, suddenly, there is a need for wide attention to other situations that require selective attention before analyzing the specific stimulus and selecting the appropriate action.

During this experiment, the illuminance pattern was kept constant, using 12 fluorescent lamps with a color temperature of 4,100 kelvin, generating a total lux measurement of ~ 822 lux, and at eye level in the vertical plane, a measurement of ~ 352 lux. According to Cajochen et al. [41], exposure to a lighting pattern of around 90 to 180 lux is sufficient to promote changes in objective and subjective measures of alertness as well as melatonin suppression. It is important to note that the research by Cajochen et al. [41] used the Constant Routine method, in which all individuals were systematically controlled in relation to the pattern of sleep, awakening, food and fluid intake, which was not possible with the same technique in our work.

Regarding the lack of positive findings under the effect of alertness/drowsiness, it is important to mention the work of Souman et al. [42], who carried out a systematic review on the acute effects of the state of alertness upon exposure to light in a review of publications of the last 26 years (1990–2016). It was found that most studies reported significant differences in subjective alertness;



► **Fig. 4** a, Accuracy results ($p=0.308$, $ES=-0.47$); b, Reaction time ($p=0.698$, $ES=-0.14$); c, T-test ($P=0.066$; $ES=0.41$). Data are expressed as individual values \pm SD.

however 17 of 45 studies (38%) were unable to find a significant effect. This can be justified by a set of factors such as chronotyping, circadian phase, history of previous light, and genetic factors. We were able to control only the chronotypology of the participants, who were classified as indifferent chronotypology (47.8 ± 9.9), and the circadian phase, with all evaluations performed in the same night period after 18:00, when the melatonin production threshold was reached in low light [24].

Regarding the state of mood, it was expected that through the use of lenses with filters, the subjective state of alertness would be changed, and consequently the state of mental sedation (alert and

► **Table 2** Correlation between melatonin differences and alertness, cognitive and performance parameters for glasses with blue and transparent lenses.

	Glasses with blue lenses	Glasses with transparent lenses
	Melatonin differences	Melatonin differences
Illuminance level	-0.432	-0.179
KSS	0.002	0.002
Anxiety	0.434	0.083
Physical sedation	-0.526 *	-0.034
Mental sedation	-0.144	0.296
Other feelings	0.066	-0.091
ANT	0.378	0.381
Reaction time	0.020	0.168
T-test	-0.429	-0.076

KSS, Karolinska Sleepiness Scale; ANT, Attentional Networks Test;
* $p=0.044$

attentive). We ascertained only an ES of 0.2 for mental sedation. Leichtfried et al. [43], unlike us, observed changes in the state of subjective mood when healthy individuals were exposed to an illumination of 6,500 kelvin in the morning. It is important to note that this color temperature pattern has a band of short length (blue spectrum), unlike the color temperature of 4,100 kelvin. This is of fundamental importance because non-visual reception is more sensitive to short wavelength bands compared to cones and rods, which are more sensitive to medium-length waves and may have influenced subjective mood. The authors also performed stimulation in the daytime, as this subjective measure was more conducive to changes in the morning.

Our objective was to observe whether stimulation during the night would modify biological circadian changes (acute melatonin suppression and subjective alertness) in conjunction with psychometric changes (subjective mood), especially in relation to mental sedation; however this it did not occur in our study. On the other hand, Plitnick et al. [44] demonstrated that night exposure to lighting with short (blue) or long (red) waves caused positive changes in the subjective measures of alertness and mood, regardless of the decrease in salivary melatonin levels. In our study, no significant correlation was found between melatonin differences and mental sedation and KSS for both glasses with blue and transparent lenses. This can demonstrate a dissociation of patterns of changes in circadian biological measures and psychological status. However, a significant negative correlation was found between melatonin differences and physical sedation.

One of the limitations of our work is related to records of sleep quality. No actigraphy measures were used to record normal sleep periods or periods of insomnia during the study. The amount and time of exposure to light is another factor that may have interfered with the results. In a clinical trial, an interesting result was identified that may point to improvements in the research methodology on the effects of exposure to light and suppression of melatonin. It has been found that exposure to blue light can increase subjective alertness, but did not influence objective alertness [45]. An impor-

tant aspect of these data is that Hanifin et al. [45] and other authors used more than 30 minutes of exposure to light [6, 7, 33]. Finally, although we measured the T-test time with a stopwatch, the considered difference measurement error between evaluators was no more than 3%.

Conclusion

Exposure to an equivalent lighting pattern using blue protection or transparent lenses (492 nm–770 nm), under the same exposure time at night, did not cause significant changes in the salivary melatonin profile, in the cognitive patterns, physical performance, and the mood state of youth volleyball players.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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