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The Effect of Circadian Photoreceptors Stimulation on the Stress Response of Subjects with High Anxiety: A Pilot Study

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Featured Application: The results obtained demonstrate the potential of utilizing the method of appropriate stimulation of the circadian system to enhance the adaptation of students with high anxiety to academic stress conditions.

Abstract: The circadian and stress-realizing systems are interconnected, and the balance of their interaction determines the state of human health. The objective of this study was to investigate the modulating effect of activating the circadian system on heart rate variability in female medical students with high anxiety while performing a cognitive task. After 20 min of adequate stimulation of circadian photoreceptors with a monochromatic blue light, the cognitive task performance resulted in a decrease in sympathetic impact. During the session of monochromatic blue light exposure, a trend of increasing heart rate variability was observed in a state of relative rest (especially in the first 5 min). A comparative analysis of the parameters of the letter cancellation test before and after light exposure revealed a statistically significant increase in the indexes reflecting mental productivity, work accuracy, and concentration of attention. The results suggest an essential physiological role of the human circadian system in modulating the autonomic and psycho-emotional conditions, as well as cognitive functions of individuals with high anxiety. Our findings indicate the possibility of quickly correcting the balance in human systemic regulatory mechanisms using the activation of retinal circadian photoreceptors by blue light.

Keywords: anxiety; circadian system; heart rate variability; melanopsin-containing retinal ganglion cells; stress

1. Introduction

Cyclical fluctuations in intensity during the day, called circadian rhythms, are observed in a wide variety of human physiological processes: from behavioral responses, motor activity, and sleep-wake cycle to cycles of hormone secretion, such as melatonin, cortisol, and thyroid-stimulating hormones. Ambient light is a major synchronizer (Zeitgeber) of

the circadian system. Daily exposure to natural light corrects the endogenous period of biological rhythms, which is not exactly 24 h, but about 24.2 h [1]. These processes are realized due to neural pathways coming from melanopsin-containing retinal ganglion cells (mRGCs), which are photoreceptors of the human circadian system. mRGCs constitute 1–2% of all retinal ganglion cells, their axons form a retinohypothalamic tract, which monosynaptically connects these cells with the center of circadian regulation, located in the suprachiasmatic nuclei of the hypothalamus (SCN). mRGCs transmit to SCN information about changes in environment illumination, due to which the cyclic change in the activity of molecular and biochemical reactions and, as a consequence, in physiological processes of the organism during the day take place [2]. The maximum sensitivity of circadian retinal photoreceptors lies in the range of the visible light spectrum with a peak wavelength of 480 ± 5 nm [3,4]. The SCN of the anterior hypothalamus serves as a central biological clock, synchronizing the peripheral clock presented in almost every tissue and organ system [5].

The evolution of the circadian and stress-realizing systems has contributed to the adaptation of the human body to repeated and spontaneous changes in the environment and to maintaining functional stability. These adaptive systems are interconnected, and the balance of their interaction determines the state of human health. It is known that stress can have a significant impact on the circadian system and can potentially lead to reversible or persistent disturbances in circadian rhythms [6]. Impaired clock gene expression, in turn, causes changes in the functioning of the hypothalamic–pituitary–adrenal axis. For example, a deficiency of BMAL1 and CLOCK proteins in mice with mutations in corresponding circadian genes (Bmal1 and Clock) results in a significant decrease in the overall level and disruption of the rhythmicity of glucocorticoid secretion [7]. In contrast, null mutations in Cryptochrome or Period genes in mice lead to an increase in glucocorticoid secretion against the background of changes in its rhythm [8,9]. A study in healthy volunteers (N = 14, age 20–41) showed that acute total sleep deprivation (for 40 h) significantly increased cortisol levels ($p < 0.0001$), while chronic circadian shift (during 25 days) significantly reduced the level of this hormone ($p < 0.05$). Thus, acute and chronic desynchronosis have opposite effects on the level of stress hormones [10]. Karatsoreos and colleagues have shown that circadian disturbances, in addition to a number of metabolic changes in animals, cause changes in the cellular morphology of the prefrontal cortex, which plays a key role in the implementation of cognitive functions. The described impairments are similar to those in chronic stress, which confirms the influence of the circadian system on neural networks that regulate cognitive activity [11].

The response of an organism to a circadian shift depends on various factors, such as the timing of experiments, characteristics of the light flux, and the initial functional state of the subject. However, the mechanisms behind the corresponding effects are not yet fully understood [12]. There is a lack of scientific publications dedicated to investigating the impact of blue light stimulation of the retinal circadian photoreceptors on the systemic mechanisms of human cardiac activity regulation, particularly considering individual stress resistance under conditions of cognitive load [13–15].

The objective of this study is to investigate the modulatory impact of circadian system activation on heart rate variability (HRV) in female medical students exhibiting high levels of anxiety during cognitive tasks.

2. Materials and Methods

The study was carried out among 18 to 20-year-old second-year students at Samara State Medical University. All the volunteers gave written informed consent to the experiment. The study was approved by the local Ethics Committee of Samara State Medical University and was conducted in accordance with the principles of the Declaration of Helsinki. The Spielberger-Hanin test and the stress resistance test were employed for subject selection. The inclusion criteria consisted of high personal (59.7 ± 1.8) and reactive (62.4 ± 2.1) anxiety levels, determined by the Spielberger-Hanin test, as well as high basic (123.5 ± 4.4) and dynamic (113.5 ± 4.8) stress sensitivity. From a pool of 465 volunteers,

17 female students who met the inclusion criteria were selected for the study. Melanopsin-containing retinal ganglion cells were stimulated using Blue Sky Pro glasses, which emit blue light (λ = 480 ± 5 nm). Blue Sky Pro glasses are designed as a narrow arc-shaped carrier, the length of which allows fixing its ends behind the auricles. Miniature containers are mounted at each end of the arc-shaped carrier, inside which a power source is located at one end of the carrier, and a control unit with a button is located at the other end of the carrier. A nose-back stop is mounted strictly in the center of the arc-shaped carrier. Inside the arc-shaped carrier, there is a channel for a cable of wires that are connected to two blocks of blue LEDs (three LEDs in each block) located in line directly opposite each eye. Light filters transmitting blue light in the spectrum of 480 ± 5 nm are fixed on the surface of the blue LEDs. The electronic control unit (relay for the number of light pauses, as well as the LED brightness control) is mounted in one container at the end of the arc-shaped carrier. Specifications of the device and modes of its use: the wavelength of the signal emitted by LEDs is 480 ± 5 nm; pause between light exposures—150–400 ms; the number of pauses between light exposures is 0–15 per minute; Illuminance (lx) and luminance (cd/m^2) modes: 765 lx, 750 cd/m^2 [16].

We assessed the stress response intensity by measuring the dynamics of 18 HRV variables, which reflect the regulatory effects of the sympathetic or parasympathetic division of the autonomic nervous system (ANS) on heart rate generation (Table 1), both before, during, and after light exposure, both in a resting state and during cognitive task performance.

Table 1. Examined parameters of HRV.

Parameter	Definition
SDNN, ms	Standard deviation of the NN-interval duration for the sample
RMSSD, ms	The square root of the sum of the squared differences in the durations of consecutive NN-intervals pairs
pNN50, %	Percentage of the adjacent NN-intervals pairs that differ in duration by more than 50 ms
HRVind, c.u.	Triangular index of heart rate variability
Heart rate, beats/min	Heart rate
Mode, ms	NN-interval duration mode
AMo, %	Amplitude of the NN-interval duration mode
DX, ms	Variation range of NN-interval duration
VLF, thousand ms^2	The power of very low-frequency (0.003–0.04 Hz) fluctuations in the duration of NN-interval
LF, thousand ms^2	The power of low-frequency (0.04–0.15 Hz) fluctuations in the duration of NN-interval
HF, ms^2	The power of high-frequency (0.15–0.40 Hz) fluctuations in the duration NN-interval
Total, thousand ms^2	The power of fluctuations in the duration of NN-interval in all ranges (0.003–0.40 Hz)
LF norm, %	The normalized value of the power of low-frequency (0.04–0.15 Hz) fluctuations in the NN-interval duration
HF norm, %	The normalized value of the power of high-frequency (0.15–0.40 Hz) fluctuations in the NN-interval duration
LF/HF	The ratio of low-frequency (0.04–0.15 Hz) and high-frequency (0.15–0.40 Hz) fluctuations in the NN-interval duration
SIM, c.u.	Intensity index of influences of the sympathetic division of the autonomic nervous system
PAR, c.u.	Intensity index of influences of the parasympathetic division of the autonomic nervous system
IB, c.u.	Stress index of regulatory systems according to R.M. Baevsky

The participants in the experiment performed the Bourdon test, also known as the letter cancellation test, online using a desktop computer. The test was administered twice, for a duration of 5 min each time, before and after the blue light exposure. This test is used to assess the level of attention, fatigue, performance, and resistance to monotonous activities that require a high level of sustained attention. The test involves tables consisting of 40 rows of letters with 40 letters in each row. The subject is required to sequentially search for and cross out the letters that appear first in each row, starting from the top row and proceeding from left to right, within the allotted time of 5 min. The limited time for performing the cognitive task contributes to stress induction. When analyzing the results of the letter cancellation test, 10 parameters were studied (Table 2).

Table 2. Examined parameters of the letter cancellation test.

Parameter	Definition	Formula
A, symbols/s	Attention speed	Number of letters viewed/operating time
T1, c.u.	Work accuracy (option 1)	Total crossed out/should have been crossed out
T2, c.u.	Work accuracy (option 2)	Correctly crossed out/should have been crossed out
T3, c.u.	Work accuracy (option 3)	Correctly crossed out/(crossed out + skipped)
E, signs	Coefficient of mental productivity	Number of letters viewed × 2nd option for work accuracy
Au, symbols/s	Coefficient of mental performance	Attention speed × ((correctly crossed out − skipped)/should have been crossed out)
K, %	Concentration of attention	Correctly crossed out/should have been crossed out
Ku, c.u.	Stability of concentration	Lines viewed × (lines viewed/(letters omitted and erroneously crossed out + 1))
V, symbols	The volume of visual information	0.5936 × number of letters viewed
Q, symbols/s	The speed of processing visual information	(Volume of visual information − 2.807 × (skipped + erroneously crossed out))/operating time

The experiments were conducted during the daylight hours (12:00–16:00). Prior to the study, the subjects were instructed to rest for 10–15 min. During the heart rate variability (HRV) registration, the subjects remained in a seated position with their eyes open. HRV was measured using photoplethysmography from the ring finger on the left hand, utilizing the ELOKS-01 pulse oximeter. HRV was recorded for 5 min during the resting state (baseline condition, Fon), for the initial 5 min of the Blue Sky Pro session (**Blue1**), and from the 15th to the 20th minute of the Blue Sky Pro session (**Blue2**). HRV registration was also performed during the letter cancellation test before (**Cor**) and after a 20-min Blue Sky Pro session (**BlueCor**) (Table 3).

Table 3. Experimental protocol.

Baseline Condition	The Letter Cancellation Test	Blue Sky Pro Session		The Letter Cancellation Test
5 min	5 min	20 min		5 min
		5 min	5 min	
HRV	HRV	HRV	HRV	HRV
Fon	*Cor*	*Blue1*	*Blue2*	*BlueCor*

Participants were instructed to sleep for at least 7 h on the eve of the experiment, and it was also prohibited to consume caffeinated beverages, alcohol, and drugs the day before the study.

Statistical data processing was performed using Statistica 12 program. The hypothesis of normal distribution was tested using Shapiro-Wilk, Kolmogorov-Smirnov, and Lilliefors tests. Parameter values with normal distribution were described by the arithmetic mean and the error of the mean. Parameter values with non-normal distribution were described by the median and quartiles. The Wilcoxon test was used to assess intragroup differences. Graphically, the data is presented in the form of "boxplot" charts, on which the upper and lower boundaries of the filled rectangle correspond to the quartiles, the horizontal line in the rectangle corresponds to the median, the "x" sign corresponds to the arithmetic mean, and round letters correspond to individual values.

3. Results

3.1. Analysis of HRV Parameters

HRV analysis was conducted both during the cognitive task and in a state of relative rest. Results showed that during the letter cancellation test before light exposure, there was an increase in HR, AMo, SIM, and IB, and a decrease in SDNN, RMSSD, HRVind, DX, PAR, and Mode compared to baseline conditions ($p < 0.05$) (Table 4). However, after the Blue Sky Pro session and during the letter cancellation test, the values of the above parameters (HR, AMo, SDNN, RMSSD, HRVind, DX, PAR, and Mode), except for SIM and IB, did not differ from the baseline values ($p > 0.08$).

Table 4. HRV study results.

Parameter	Condition [1]					Comparison [2] (p_0)		
	Fon	Cor	Blue1	Blue2	BlueCor	Fon vs. Cor	Fon vs. BluCor	Cor vs. BluCor
SDNN, ms	61.6 ±2.9	55.1 ±3.7	61.7 ±4.6	62.4 ±3.4	56.9 ±3.3	0.049	0.136	0.379
RMSSD, ms	50 (38; 60)	38 (29; 54)	48 (36; 63)	45 (38; 67)	42 (36; 69)	0.044	0.177	0.044
NN50, ms	86 (58; 127)	49 (29; 117)	92 (44; 124)	81 (46; 145)	57 (45; 155)	0.080	0.276	0.055
HRVind, c.u.	11.9 ± 0.6	10.2 ± 0.7	11.1 ± 0.7	12.1 ± 0.7	10.5 ± 0.6	0.023	0.079	0.351
Moda, ms	730 (680; 810)	680 (620; 740)	750 (730; 830)	740 (690; 800)	710 (690; 800)	0.002	0.394	0.002
AMo, %	7.8 ± 0.4	9.5 ± 0.6	8.8 ± 0.7	7.7 ± 0.5	9.0 ± 0.4	0.016	0.081	0.209
DX, ms	350 (330; 390)	330 (260; 390)	360 (310; 390)	310 (300; 370)	330 (310; 380)	0.047	0.281	0.551
Heart rate, bpm	79.7 ± 2.4	84.2 ± 2.7	78.2 ± 2.3	78.2 ± 2.3	79.7 ± 2.4	0.002	0.778	0.001
VLF, thousand ms^2	2.0 (1.2; 3.1)	1.5 (1.0; 2.8)	2.9 (1.5; 3.4)	2.7 (1.6; 5.2)	1.6 (1.1; 2.3)	0.523	0.266	0.868
LF, thousand ms^2	3.9 (2.4; 4.3)	2.1 (1.7; 3.4)	2.6 (1.6; 3.9)	4.6 (2.1; 6.1)	2.3 (2.2; 2.9)	0.025	0.035	0.554
HF, thousand ms^2	3.2 (1.4; 4.8)	1.4 (0.8; 2.7)	2.9 (0.9; 3.6)	2.1 (1.5; 3.5)	1.6 (0.9; 2.5)	0.006	0.044	0.332
Total, thousand ms^2	9.5 ± 1.1	6.5 ± 0.7	9.5 ± 1.5	10.0 ± 1.2	6.9 ± 0.8	0.007	0.019	0.554
LF norm, %	55.1 ± 3.8	57.7 ± 3.5	58.1 ± 3.7	60.8 ± 3.5	57.9 ± 4.5	0.523	0.831	0.868
HF norm, %	44.9 ± 3.8	42.3 ± 3.5	41.9 ± 3.7	39.2 ± 3.5	42.1 ± 4.5	0.523	0.831	0.868
LF/HF	1.02 (0.81; 2.04)	1.30 (0.93; 1.99)	1.18 (0.86; 2.11)	1.53 (1.08; 2.57)	1.53 (0.68; 3.03)	0.796	0.758	0.943
SIM, c.u.	2.3 ± 0.3	3.6 ± 0.4	2.9 ± 0.5	2.4 ± 0.3	3.0 ± 0.3	0.006	0.006	0.049
PAR, c.u.	14.6 ± 0.8	12.0 ± 0.9	13.7 ± 0.9	14.5 ± 0.9	13.1 ± 0.8	0.031	0.093	0.332
IB, c.u.	71 ± 7	110 ± 13	81 ± 10	73 ± 8	86 ± 9	0.006	0.049	0.028

[1]—With a normal distribution, the mean and standard error of the mean were calculated, with a distribution other than normal, the median and quartiles were calculated; [2]—p_0 calculated by Wilcoxon Matched Pairs Test.

When comparing HRV during the letter cancellation test before and after the Blue Sky Pro session, the following trends were observed (Figure 1): after the Blue Sky Pro session, HR values decreased by 4.5 ± 1.1 beats/min (p = 0.001), SIM decreased by 0.6 ± 0.3 c.u. (p = 0.049), IB decreased by 24 ± 8 c.u. (p = 0.028), and the values of Mode and RMSSD increased by 43 ± 11 ms (p = 0.002) and 5.3 ± 2.5 ms (p = 0.044), respectively.

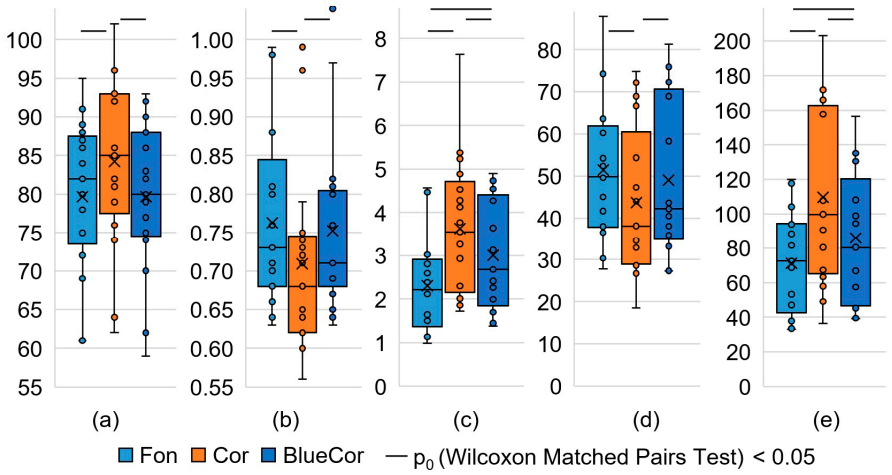

Figure 1. HRV parameters during the cognitive task before (Cor) and after the Blue Sky session (BlueCor): (**a**) HR, bpm—heart rate; (**b**) Moda, ms—NN-interval duration mode; (**c**) SIM, c.u.—intensity index of influences of the sympathetic division of the autonomic nervous system; (**d**) RMSSD, ms—the square root of the sum of the squared differences in the durations of consecutive NN-intervals pairs; (**e**) IB, c.u.—stress index of regulatory systems according to R.M. Baevsky.

The HRV spectral analysis results during the letter cancellation test, both before and after light exposure, showed a decrease in **LF**, **HF**, and **Total** compared to the baseline condition (p < 0.05). However, a comparative analysis of HRV spectrogram parameters during cognitive tasks before and after the Blue Sky Pro session did not reveal any statistically significant differences (p > 0.3). In the resting state during the Blue Sky Pro session, the intragroup HRV analysis showed the following trends: during the first 5 min of light exposure (Blue1), **HR** and **HF** values decreased by 1.5 ± 0.7 beats/min and 753 ± 218 ms^2, respectively, compared to the baseline, while **Mode** increased by 20 ± 9 (p < 0.05) (Figure 2).

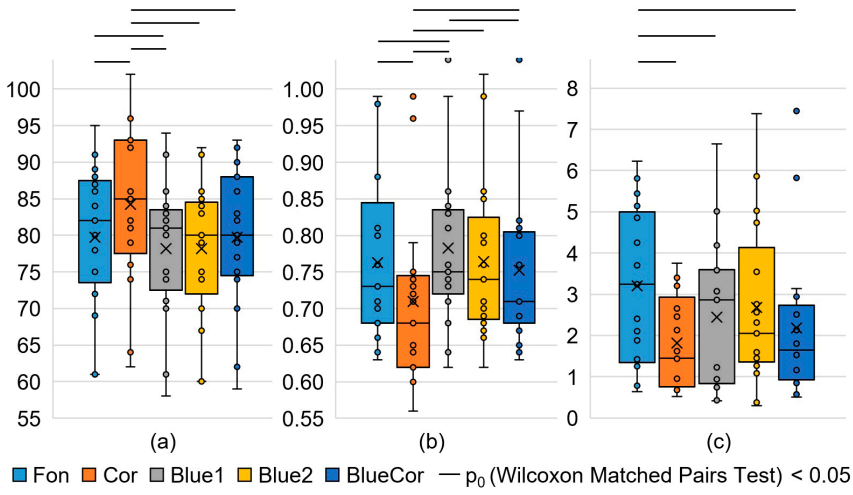

Figure 2. HRV parameters reflecting statistically significant differences between the study stages: (**a**) HR, bpm—heart rate; (**b**) Moda, ms—NN-interval duration mode; (**c**) HF, thousand ms^2—the power of high-frequency (0.15–0.40 Hz) oscillations in the NN-interval duration.

The aforementioned patterns depict a decline in the intensity of sympathetic nervous system influences at the start of the Blue Sky Pro session. Examining HRV during the last five minutes of the 20-min light exposure (Blue2) did not exhibit any statistically significant changes in HRV in comparison to the baseline ($p > 0.09$).

3.2. Analysis of the Cognitive Task Results

A comparative analysis of the parameters of the letter cancellation test was conducted before and after the Blue Sky Pro session. The results showed a statistically significant increase in the following indices: speed of attention (**A**) by 0.39 ± 0.11 symbols/s, work accuracy (**T1** and **T2**) by 0.07 ± 0.02 c.u., coefficient of mental productivity (**E**) by 149 ± 42 symbols, coefficient of mental performance (**Au**) by 0.70 ± 0.20 symbols/s, concentration of attention (**K**) by 6.9 ± 2.2%, volume of visual information (**V**) by 45.8 ± 15.2 symbols, and speed of processing visual information (**Q**) by 0.26 ± 0.05 symbols/s ($p < 0.05$) (see Table 5 and Figure 3). The observed changes in the parameters of attention and the speed of information processing after light exposure may be the result of the activation of circadian photoreceptors.

Table 5. The results of the letter cancellation test.

Parameter	Condition [1]		Comparison (Wilcoxon Matched Pairs Test)		
	Cor	BlueCor	W	Z	p_0
A, symbols/s	4.7 ± 0.3	5.1 ± 0.3	18	2.769	0.006
T1, c.u.	0.84 (0.69; 0.94)	0.92 (0.83; 0.93)	24	2.485	0.013
T2, c.u.	0.84 (0.69; 0.92)	0.91 (0.82; 0.93)	23	2.533	0.011
T3, c.u.	0.94 (0.91; 0.96)	0.95 (0.91; 0.97)	58	0.876	0.381
E, symbols	1216 (853; 1439)	1430 (1314; 1446)	21	2.627	0.009
Au, symbols/s	3.5 ± 0.4	4.2 ± 0.3	5	3.385	0.001
K, %	84 (69; 92)	91 (82; 93)	20	2.675	0.007
Ku, c.u.	160 (97; 200)	169 (123; 320)	42	1.633	0.102
V, symbols	890 (773; 943)	925 (865; 946)	22	2.379	0.017
Q, symbols/s	2.7 ± 0.2	3.0 ± 0.1	9	3.195	0.001

[1]—With a normal distribution, the mean and standard error of the mean were calculated, with a distribution other than normal, the median and quartiles were calculated. **Cor**—performing the letter cancellation test before Blue Sky Pro session; **BlueCor**—performing the letter cancellation test after 20-min Blue Sky Pro session.

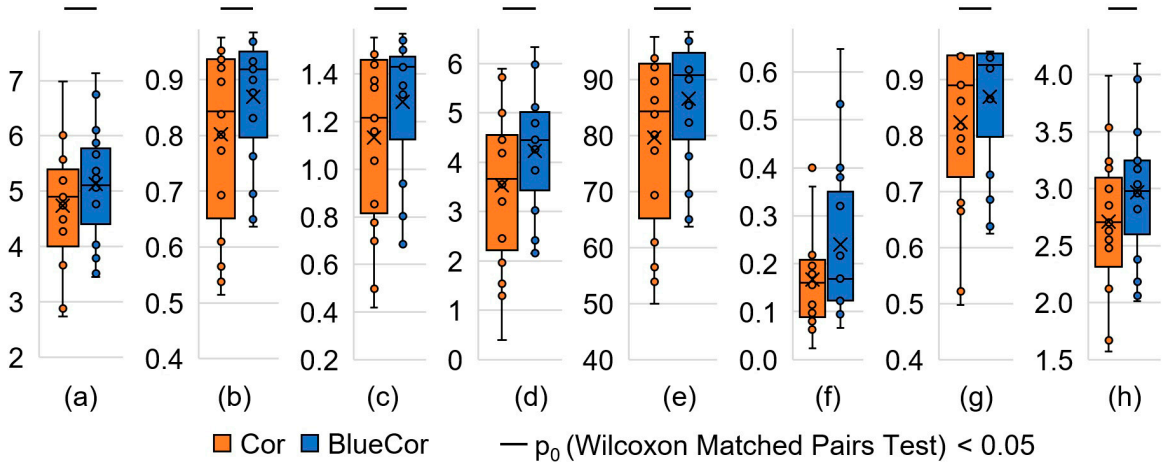

Figure 3. The letter cancellation test parameters before (Cor) and after the Blue Sky session (BlueCor): (**a**) A, symbols/s—Attention speed; (**b**) T1, c.u.—option 1 work accuracy; (**c**) E, thousand symbols—the coefficient of mental productivity; (**d**) Au, symbols/s—the coefficient of mental performance; (**e**) K, %—concentration of attention; (**f**) Ku, c.u—stability of concentration; (**g**) V, symbols—the volume of visual information; (**h**) Q, symbols/s—the speed of processing visual information.

The results obtained indicate the possibility of modulating autonomic processes and cognitive functions in women with high anxiety by using adequate stimulation of mRGCs.

4. Discussion

Numerous studies have shown that HRV is an indicator of the degree of tension in regulatory systems in response to any stress impact that activates the Hypothalamic–pituitary–adrenal and sympathoadrenal systems. Analysis of HRV provides information about changes in autonomic tone due to the influence of physiological and psychological factors, making HRV measurements useful in monitoring sleep, stress, sleepiness, and physical training parameters [17].

Prior to light exposure, the cognitive task activated the central circuit in subjects with high anxiety, resulting in a shift towards sympathetic dominance of the sympathetic-vagal balance (increase in IB). This was reflected in the stabilization of heart rate, a decrease in the variability of cardiac intervals, and an increase in the number of intervals with the same duration (increase in AMo) [18]. The decrease in HRV observed in this study (a sharp decrease in SDNN and total power of oscillations, and a decrease in NN interval duration) reflects the disruption of regulatory and homeostatic functions of the autonomic nervous system in response to stress, reducing the organism's ability to cope with internal and external stressors [19]. Dimitriev et al. also reported that cognitive stress leads to increased predictability, regularity of RR intervals, and decreased HRV [20]. Under stress, more stable and periodic heart rate fluctuations and inhibition of parasympathetic activation increase human vulnerability to stress [19]. The baseline functional state of subjects with high anxiety, characterized by the predominance of low-frequency fluctuations in the HRV spectrogram, may have been a prerequisite for such an HRV response. This is supported by literature indicating that high HRV is associated with a tendency to constructively perceive signals, a willingness to approach new objects and faster disappearance of fear [21,22]. Anxiety is a negative emotional reaction that causes autonomic reactivity to a stressful situation with disproportionately higher intensity than the objective danger. The neuronal organization of anxiety involves the amygdala-prefrontal circuit [23]. Neuroimaging studies have shown that anxiety disrupts the recruitment of prefrontal control mechanisms when interpreting emotionally ambiguous stimuli, resulting in amygdala hyperreactivity [23]. Thayer J.F. argued in his meta-analysis the possibility of using HRV as a marker of functional integration between vmPFC and the brainstem, as well as flexible control of vmPFC over the ANS [22].

Research-based on a multiscale entropy approach to studying functional systems has shown that entropy is a relevant marker of stress-induced changes in heart—brain interactions, even under conditions of mild stress. A degradation of cardiac entropy reflects the neurovisceral integrated response to anxiety during a cognitive task [24]. Neuroimaging studies on anxiety and stress have shown a decrease in activity in the prefrontal and anterior cingulate cortex along with an increase in amygdala activity. This leveling of top-down control and connections between these structures leads to less adaptive and flexible autonomic control of the heart and, accordingly, a loss of cardiac entropy in humans with high anxiety during cognitive tasks. However, cardiac entropy can also be a marker of increased complexity and adequate self-regulation during a cognitive task. A decrease in the entropy of the heart output signals may reflect an overflow of neural information, which may be associated with amygdala-induced impairment of cortical-subcortical processing in anxious individuals [24]. This trend is supported by studies of psychopathological conditions, such as anxiety, depression, post-traumatic stress disorder, and schizophrenia, which are associated with prefrontal hypoactivity and a lack of inhibitory neural processes. This is reflected in a deficit of working memory and executive function, poor adaptation to new neutral stimuli, and failure to recognize security signals, a bias against threat information, inefficient processing and regulation of affective information [25].

In the present study, we found that 20 min of activation of the circadian system caused a decrease in sympathetic impact during the cognitive task. Heart rate and IB

decreased, while Mode and RMSSD, an activity indicator of the parasympathetic link of autonomic regulation, which reflects the performance of the autonomic regulation circuit and is characterized by high-frequency fluctuations, increased. A similar trend of increasing HRV was also observed in a state of relative rest during the Blue Sky Pro session, especially in the first 5 min. The phenomenon of HRV increase during activation of the circadian system, which we discovered, is also confirmed by other studies. Daniela Litscher et al. stimulated the retina of healthy volunteers with a light flux of 456 nm for 10 min in the morning (9:00–11:00). In the second half of the blue light stimulation session, as well as in the subsequent 5 min, a significant decrease in heart rate was observed ($p < 0.05$). The total spectrum power of HRV also decreased ($p = 0.029$) during light exposure [26].

In individuals with high-stress sensitivity, the observed decrease in the sympathetic activity of the autonomic nervous system (ANS) during stimulation of circadian retinal photoreceptors can be explained by the numerous projection connections between the circadian and stress-realizing systems. The human stress-realizing system consists of central and peripheral components. The central components are mainly located in the hypothalamus and the brain stem and include neurons of the hypothalamic paraventricular nuclei, the paragigantocellular and parabrachial nuclei of the medulla, and the locus coeruleus, the arcuate nucleus, noradrenergic groups of cells in the brain stem and pons, and the central nuclei of the ANS. These loci interact with each other, influencing their own activity, and also affect other brain subsystems, such as the mesocortical/mesolimbic dopaminergic system, and the central nuclei of the amygdala [6]. Under chronic stress, the brainstem and the limbic system receive corresponding signals from sensory systems and transmit these signals to the paraventricular nucleus of the hypothalamus, where the secretion of corticotropin-releasing hormone and arginine vasopressin is stimulated [27].

The peripheral components of the stress system include the hypothalamic-pituitary-adrenal axis and the ANS, which largely complement each other. The regulation of the hypothalamic-pituitary-adrenal axis partly depends on the ANS, especially on the vagus nerve influences [6]. Controlling the links of the hypothalamus-pituitary-adrenal axis by the circadian clock is realized at several levels. Neurons of the suprachiasmatic nuclei (SCN) form projections to the hypothalamic structures, modulating the rhythmic activity of the underlying neural networks [28]. There are direct and indirect neuronal projections (through the subparaventricular zone and the dorsomedial nucleus of the hypothalamus) of the SCN on the corticoliberin- and vasopressin-secreting neurons of the medial parvocellular paraventricular nucleus of the hypothalamus [6,28]. These projections modulate the circadian rhythm of corticoliberin and vasopressin secretion and, accordingly, the rhythmic secretion of adrenocorticotropic hormone (ACTH) by the pituitary gland [29]. SCN neurons also transmit information via multisynaptic autonomic innervation (preganglionic neurons of the lateral horns in the spinal cord and splanchnic nerves) to the adrenal medulla, and then via catecholamines to the adrenal cortex, thus modulating time-of-day-dependent sensitivity of the zona fasciculata to ACTH [27] and regulating the circadian rhythm of glucocorticoid release under exposure to light independently of the hypothalamic–pituitary–adrenal axis through direct interaction with the peripheral circadian clock in the adrenal glands (i.e., regulation of PER1 and StAR gene expression) [6].

The circadian clock controls several links of the hypothalamic–pituitary–adrenal axis, leading to increased reactivity during the active phase of circadian rhythms. Cortisol and corticosterone exhibit stable circadian fluctuations with peak concentration in the blood shortly before the onset of the circadian active phase, typically in the early morning for humans [30]. Additionally, the Hypothalamic–pituitary–adrenal axis modulates mood regulation, with rhythms generated in the SCN potentially changing glucocorticoid levels in response to stress [31]. Circadian fluctuations also influence human cardiovascular parameters, such as heart rate, blood pressure, baroreflex, heart rate variability, and plasma adrenaline and noradrenaline levels, demonstrating a peak in sympathetic activity and the lowest parasympathetic activity in the morning hours [32].

Our study found that 20-min light stimulation of melanopsin-containing ganglion cells increased HRV and parasympathetic autonomic regulation in students with high anxiety performing a cognitive task. This increase reflects an expansion of adaptive potential and improved efficiency in completing the task due to heightened attention and visual processing speed. Our results are supported by fMRI studies showing that short-term (18 min) exposure to blue light with a 470 nm wavelength during an auditory working memory task increases brain activity in areas associated with working memory and attention, including the frontal and parietal lobes of the cerebral cortex and the thalamus, compared to greater wavelength light (550 nm) [33]. Blue light has also shown efficacy in treating insomnia and hypersomnia disorders such as narcolepsy and idiopathic hypersomnia [34].

5. Limitations

We acknowledge at least three limitations of this study. First, the results may be affected by the small sample size and gender homogeneity. The reviews [35,36] describe studies demonstrating gender differences in stress response from the molecular to the systemic level. Sex differences in SCN neurochemistry and differences in androgen and estrogen receptors in SCN in both spatial and temporal expression patterns have been reported in human and rodent models [37]. Accordingly, we can expect to find gender differences in circadian system activation under cognitive testing conditions. Second, the present study did not include a comparison group (a group of subjects prone to anxiety who would repeatedly perform a cognitive test without 20-min adequate stimulation of the circadian system) and a control group (a group of subjects not prone to anxiety). Third, we did not control subjects' anxiety before the first and second cognitive testing. In our study, subjects prone to test anxiety participated, so we can assume that their anxiety was higher when they first performed the test, compared to the second test after the light exposure. The use of a cross-sectional experimental design would eliminate this limitation.

Using a cross-sectional study design, expanding the sample of subjects, and including comparison and control groups in future studies will provide a deeper understanding of the effects of circadian system activation on the psycho-emotional state of students with high anxiety during cognitive tasks.

6. Conclusions

Our findings suggest that it is possible to rapidly adjust the autonomic and psycho-emotional state of an individual by appropriately activating circadian photoreceptors. This opens up opportunities for developing a methodology to optimize the functional state of students and improve the effectiveness of the educational process.

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