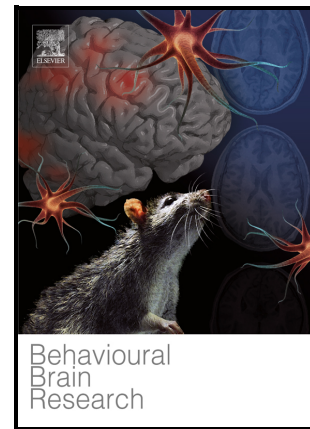


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Cerebral, Systemic Physiological and Behavioral Responses to Colored Light Exposure during a Cognitive Task: A SPA-fNIRS Study

Hamoon Zohdi ^{1,2,*}, Josefa Märki ¹, Felix Scholkmann ^{1,2} and Ursula Wolf ¹

¹ Institute of Complementary and Integrative Medicine, University of Bern, 3012 Bern, Switzerland

² Biomedical Optics Research Laboratory, Department of Neonatology, University Hospital Zurich, University of Zurich, 8091 Zurich, Switzerland

*Correspondence: hamoon.zohdi@unibe.ch

Institute of Complementary and Integrative Medicine, University of Bern, Fabrikstrasse 8, 3012 Bern, Switzerland

Abstract: Colored light has important implications for human health and well-being, as well as for the aesthetics and function of various environments. In addition to its effects on visual function, colored light has significant effects on cognitive performance, behavior and systemic physiology. The aim of the current study was to comprehensively investigate how colored light exposure (CLE) combined with a cognitive task (2-back) affects performance, cerebral hemodynamics, oxygenation, and systemic physiology as assessed by systemic physiology augmented functional near-infrared spectroscopy (SPA-fNIRS). 36 healthy subjects (22 female, 14 male, age 26.3 ± 5.7 years) were measured twice on two different days. They were exposed to the sequence of blue and red light or vice versa in a randomized crossover design. During the CLE, the subjects were asked to perform a 2-back task. The 2-back task performance was correlated with changes in the concentration of oxygenated hemoglobin in the prefrontal cortex (red: $r = -0.37$, $p = 0.001$; blue: $r = -0.33$, $p = 0.004$) and the high-frequency component of the heart rate variability (red: $r = 0.35$, $p = 0.003$; blue: $r = 0.25$, $p = 0.04$). These changes were independent of the CLE. Sequence-dependent effects were observed for fNIRS signals at the visual cortex (VC) and for electrodermal activity (EDA). While both colors caused relatively similar changes in the VC and EDA at the position of the first exposure, blue and red light caused greater changes in the VC and EDA, respectively, in the second exposure. There was no significant difference in the subjects' 2-back task performance between the CLE ($p = 0.46$). The results of this study provide new insights into how human physiology and behavior respond to colored light exposure. Our findings are important for understanding the impact of colored light in our daily lives and its potential applications in a variety of settings, including education, the workplace and healthcare.

Keywords: systemic physiology augmented functional near-infrared spectroscopy; SPA-fNIRS; colored light exposure; 2-back task; cerebral hemodynamics; systemic physiology; task performance

1. Introduction

Light is not only essential for us humans to perceive our environment, but it is also a major factor influencing a wide range of physiological and behavioral parameters [1–6]. While control over daylight is limited, artificial colored light can be adjusted to support human health and well-being, i.e. psychological and physiological functioning, through both visual and non-visual pathways [7,8]. Among various non-visual functions, colored light has been shown to be an effective non-contact modulator of cognitive performance [9,10]. Improving cognitive performance with colored light may be useful for educational purposes. For example, it has been shown that the lighting conditions in the school have a positive influence on students' concentration depending on the light intensity (between 300 and 1000 lux) and correlated color temperature (CCT, between 3000 and 12000 K) [11]. It was found that students in the “Focus” lighting setting (illuminance: 1000 lux, CCT: 6500 K) performed better on the concentration task and made fewer errors than their peers in the “Normal” lighting setting (illuminance: 300 lux, CCT: 3000 K).

The n-back task is a cognitive task commonly used in psychology and cognitive neuroscience to assess working memory [12]. This task is usually either auditory or visual and consists of presenting a series of stimuli. Participants are asked to respond to the stimuli they have heard or seen with “n” items back [13]. Since the 1960's, the n-back task has been used in various fields such as psychology, behavioral neuroscience and neuroimaging research. In particular, in the field of neuroimaging, several studies have investigated working memory and brain function using n-back tasks [14–16]. Functional near-infrared spectroscopy (fNIRS) is a neuroimaging technique, which is based on measuring changes in the concentrations of oxygenated ($[O_2Hb]$) and deoxygenated ($[HHb]$) hemoglobin associated with neuronal activity in cerebral cortex [17,18]. Since the changes in $[O_2Hb]$ and $[HHb]$ are also influenced by other physiological factors, primarily changes in systemic physiology, these factors must be measured simultaneously [19–22]. Therefore, the systemic physiology augmented fNIRS (SPA-fNIRS) method has been developed and is now increasingly used [23–25]. SPA-fNIRS is a powerful and holistic approach to study the physiological state of a person and the interaction between body and brain, allowing a deeper understanding of the fNIRS signals and avoiding misinterpretations [23,26].

In our previous research, using SPA-fNIRS, we reported effects of colored light on physiological measures and verbal fluency performance [27–30]. More research is needed to clarify the effects when complex tasks that challenge working memory (e.g. n-back tasks) are used [31]. The reason for linking a cognitive task with colored light exposure (CLE) is to investigate the effects of colored LED lights for learning contexts. Colors and colored lights are optional design elements of physical learning environments and can have a variety of effects on behavior and learning in educational

settings. Therefore, the aim of the current study is to investigate with SPA-fNIRS whether and how colored light exposure (CLE with blue and red) and a 2-back task affect cerebral hemodynamics, oxygenation and systemic physiology and how task performance is affected by blue and red light exposure.

2. Materials and Methods

2.1. Participants

Thirty-six healthy right-handed subjects (22 women, 14 men, age 26.3 ± 5.7 years) volunteered to participate in this study. The sample size was calculated by a power analysis to detect substantial effects (effect size: $d = 0.59$; based on our previous study results investigating the different physiological effects of long-term colored light exposure with two colors) at a $p < 0.05$ and a power of > 0.8 . Handedness was determined by the Edinburgh Handedness Inventory [32]. The subjects were all non-smokers, free of medication and without diagnosed sleep, neurological and psychiatric disorders or alcohol/drug abuse. Subjects were asked not to consume caffeine or food for 2 h before the experiment. All subjects signed a written informed consent form prior to the measurements and were financially compensated for their participation at the end of the measurements. The study was approved by the Ethics Committee of the Canton of Bern, and was conducted and documented in accordance with the fNIRS guidelines [33]. We protected participants' privacy and well-being throughout the study.

2.2. Light exposure

Six stage lights (RUSH PAR 2 RGBW Zoom™, Martin by Harman, Arhus, Denmark) were used to shine blue and red light into a half chamber with three white walls and a ceiling around a subject (each wall: height: 3 m, width: 2.5 m). CLE varied in terms of color (red vs blue), but duration and intensity were fixed. Each stage light has a matrix of 12 RGBW light-emitting diodes (LEDs). The peak wavelengths of the blue and red LEDs were ~ 450 nm (full width at half maximum, FWHM ~ 20 nm) and ~ 640 nm (FWHM ~ 20 nm), respectively. Illumination was set to 120 lux for both colors using a DT-1308 light meter (ATP Instrumentation Ltd., UK) at eye level.

2.3. Measurement protocol

All subjects were exposed to two colored light conditions on two different days, but at the same time of day. They sat upright and looked at a white wall of a half chamber (distance: ~ 160 cm) illuminated by six stage spotlights. Subjects were exposed to the sequence of blue followed by

red light or vice versa in a randomized crossover design. Each measurement lasted 35 minutes (5 periods of 7 minutes each, Figure 1a). In the beginning, the subjects were in the darkness (baseline; No-light phase) for 7 minutes followed by 28 minutes of CLE, i.e., 14 min color 1, 14 min color 2. The CLE period was divided into 7 min 2-back tasks under color 1, 7 min recovery (color 1), 7 min 2-back tasks under color 2, and 7 min recovery (color 2). We conducted baseline measures as a control condition at the beginning of measurement without exposure to colored light. This allows for comparisons between baseline and CLE phases for cerebral and systemic physiological parameters. During the CLE, subjects were asked to perform a 2-back task. In this task, a series of consonants (c, h, k, l, q, r, s, t) were presented auditorily and subjects had to respond as quickly as possible by pressing a key each time the current letter was identical to the one that was presented before last (i.e., 2 positions back in the sequence). Each letter was presented for 500 ms, with a 3000 ms interval between letters.

The 2-back task was presented in a standardized and consistent manner to all subjects, ensuring that the task's difficulty remained constant across conditions. The 2-back task phases consisted of three different trials (2 min each) with a 30-second break in between. Each trial consisted of 40 letters presented in random order. 10 ± 3 of the letters presented in each trial corresponded to the target stimulus ("match" response). In the following 7 minutes of the first color, the subjects were asked to relax (recovery phase). In the last 14 minutes of the measurement, the same procedure was repeated with a second, different colored light exposure (red or blue).

2.4. Physiological recording

The SPA-fNIRS method comprises measuring simultaneously several fNIRS along with systemic physiological parameters: (i) Absolute values of $[O_2Hb]$, $[HHb]$, total hemoglobin ($[tHb]$) and tissue oxygen saturation (StO_2) measured with a multi-channel frequency-domain near-infrared spectroscopy (FD-NIRS) instrument (Imagent, ISS Inc, Champaign, IL, USA) in the prefrontal (PFC) and visual (VC) cortex at a sampling rate of 2.5 Hz. The light source of the ISS Imagent consists of 16 laser diodes at 760 nm and 16 laser diodes at 830 nm. Four highly sensitive photomultiplier tubes serve as detectors. Each of the four ISS sensors had four light emitters and one light detector connected to an optical fiber delivering the light to the photomultiplier tube. The source-detector separations of the optodes were 2.0, 2.5, 3.5, and 4.0 cm over the PFC and 2.0, 2.5, 3.0, and 3.5 cm over the VC. The FD-NIRS measurement allows to determine absolute values of $[O_2Hb]$, $[HHb]$, $[tHb]$ and StO_2 by directly measuring the absorption and scattering properties of the tissue. The device implements a self-calibration measurement approach which enables a depth-sensitive measurement (being less influenced by extracerebral hemodynamic changes)

and is less sensitive to movement artifacts [34]. (ii) End-tidal carbon dioxide (P_{ETCO_2}) and respiration rate (RR) at a sampling rate of 1 Hz by NONIN LifeSense (NONIN Medical, Plymouth, MN, USA). (iii) Arterial oxygen saturation (SpO_2), mean arterial blood pressure (MAP), heart rate (HR) and low-frequency (LF, 0.04–0.15 Hz) and high-frequency (HF, 0.15–0.4 Hz) components of the heart rate variability (HRV) with SOMNOtouch NIBP (SOMNOmedics GmbH, Randersacker, Germany) at a sampling rate of 1 Hz. (iv) Skin conductance level (SCL) at a sampling rate of 8 Hz (VERIM system Mind-Reflection, Tallinn, Estonia). (v) Finally, to measure the coupling between HR and RR, the pulse-respiration quotient (PRQ) was calculated ($PRQ = HR/RR$) [35,36]. All data were recorded concurrently. Figure 1b and 1c show the positions of the devices and sensors on the test subject as well as the experimental setup with the measurement devices, the illuminated walls and the stage lighting.

2.5. Data processing

Signal processing and data analysis were performed in MATLAB (R2022a, MathWorks, Inc., Natick, MA, USA). Prior to analysis, extensively noisy and unreliable data (e.g. extremely low ($P_{ETCO_2} < 20$ mmHg) or unreasonably high values ($StO_2 > 100\%$)) were removed by manual inspection. Missing values in time series data were identified by summary statistics and specific functions in MATLAB (e.g. “ismissing” function). Then, they were filled by using the forward/backward fill, i.e. “nearest” function in MATLAB. The proportion of missing values was 0.3% and in general, 92% of time series are of sufficient quality for data processing. fNIRS signals were low pass filtered using a robust 2nd-degree polynomial moving average filter (RLOESS) with a window length of 1 min. Signals from the left and right PFC and VC were then averaged to obtain signals for the whole PFC and VC, respectively. Systemic physiological signals, except for SCL, were also smoothed by the RLOESS filter with a window length of 1 min. The electrodermal activity data were processed using the Ledalab toolbox [37,38], and the low frequency (tonic) component of the skin conductance was extracted as SCL by continuous decomposition analysis.

2.6. Statistical analysis

All analyses were performed using MATLAB and JASP (jasp-stats.org, version 0.14.1.0). The normal distribution of all parameters was tested using the Shapiro-Wilk test. In the case of normally distributed data, the parametric methods were applied. Wilcoxon signed-rank and Mann-Whitney tests were used when homogeneity of variance or normality of distribution between different colors/conditions was violated. The magnitude of the difference for each comparison was assessed

by Cohen's *d*. i.e., small (0.2), medium (0.5), or large (0.8). Statistical significance was set at $p < 0.05$ for most analyses. The values are presented as median \pm standard error of median (SEM).

Task performance: At the behavioral level, we collected data on subjects' cognitive performance, including accuracy, i.e., net score. We recorded the number of correct (#Hits) and incorrect (#FalseAlarms) responses of the subjects. A net difference score was calculated from these two values (Net score = #Hits – #FalseAlarms). This index reflects how well the subjects were able to distinguish between correct and incorrect answers during the 2-back task [39,40]. The Wilcoxon signed-rank test was used to determine the significance of the task performance between the blue and red light exposure.

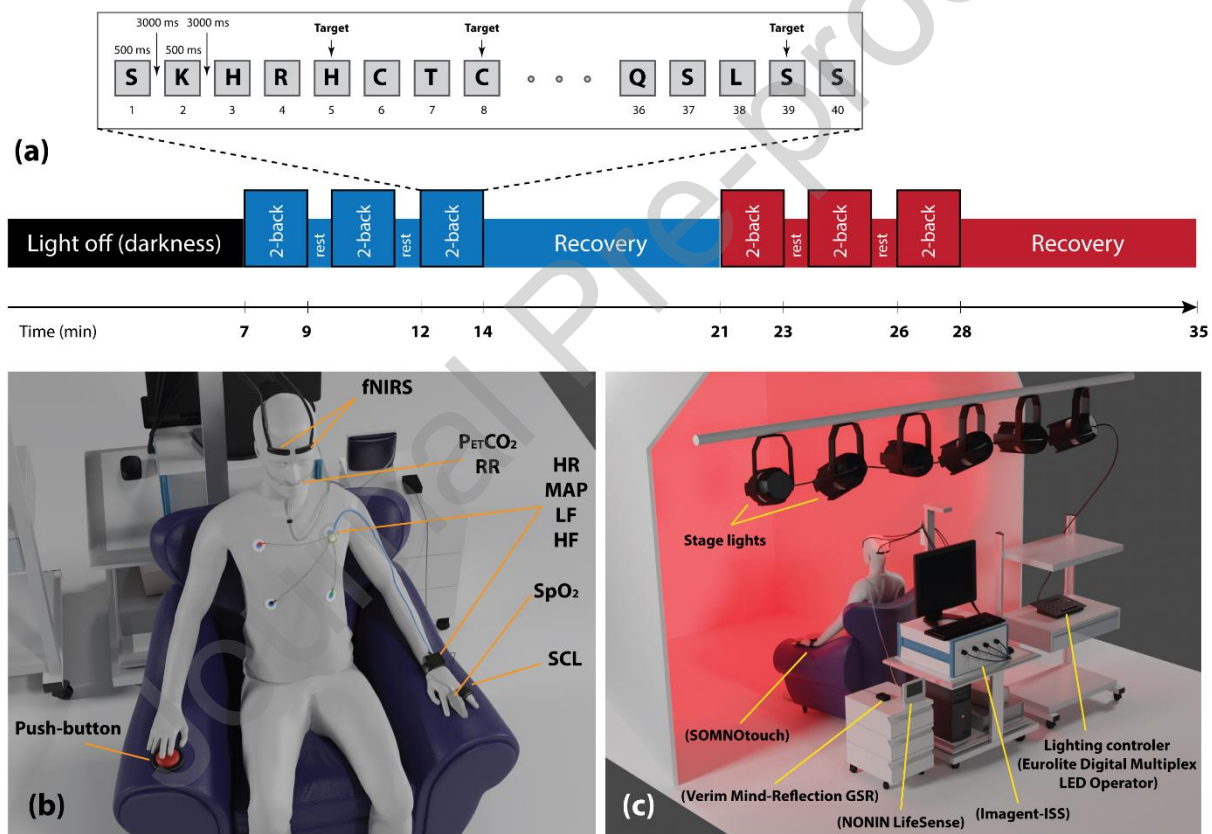


Figure 1. (a) The experimental paradigm with an example of a 2-back task. (b) The sensors/devices attached to the subject. (c) Experimental setup with the position of measurement instruments, the subject, stage lighting and the illuminated walls and the ceiling of the half chamber.

Cerebral and physiological parameters: All signals were segmented into 14 parts with a length of 140 seconds each, starting with the first segment 140 seconds after the beginning of the experiment. The median of each segment was calculated. Two segments were assigned to the “No-light” phase and three segments for each of the other phases. The median of each segment was then normalized to the last time point of the “No-light” phase. The median of each segment

was then calculated for all subjects to determine the changes in all parameters at the group level. Statistical significance was set at $p < 0.05$ for most analyses. In addition, a Wilcoxon signed-rank test was used for each time point compared to the last time point of the “No-light” phase, and a false discovery rate (FDR) correction following the Benjamini and Hochberg [41] procedure was applied to correct for the multiple comparison situation. FDR with a q -value of 0.05 was used as the threshold. A cluster-based permutation test was applied to identify differences between conditions (blue-red vs. red-blue) for all parameters while accounting for multiple comparisons (p -values = 0.05; Number of permutations = 15000). To compare the effects of the two conditions on the changes in cerebral and systemic physiological signals during the task and recovery phases, each time point with blue-red light was compared with the same time point with red-blue light using the Wilcoxon-Mann-Whitney test. Moreover, to compare the effects of both colored lights on the changes in all signals, each time point of red was compared to the same time point of blue within one condition using the Wilcoxon signed-rank test. In addition, the area under the curve (AUC) of the 2-back/CLE phases for each parameter was calculated for each subject for both conditions (blue-red and red-blue), and Pearson’s correlation coefficients were calculated based on the AUC values to examine the bivariate relationship between all cerebral and systemic physiological parameters as well as task performance.

3. Results

Figure 2a shows the net scores of the 2-back tasks performed for subjects exposed first to blue followed by the red light (first participation) and then to red followed by the blue light (second participation). Significant differences were observed between the net score under blue light during the first participation and the net scores for both color conditions during the second participation (blue 1st vs red 2nd: $p < 0.01$; effect size (Cohen’s d): $d = 0.7$; blue 1st vs blue 2nd: $p < 0.01$; $d = 0.8$). No significant differences were found for the net scores of subjects exposed to red light followed by blue light at the first participation and blue light followed by red light at the second participation (Figure 2b). Figure 2c and Figure 2d show the combination of the net scores of each group for exposure to blue and red light, i.e. blue (1st and 2nd) vs red (1st and 2nd). In both cases, no significant difference in task performance was observed between blue and red light. A summary of task performance for all subjects, regardless of light condition and subject group, is shown in Figure 2e. No statistically significant difference was found in the net score of the 2-back task performance between blue and red light exposure (blue: 89 ± 11 (mean \pm SD), range: 54-100; red: 90 ± 10 , range: 53-100).

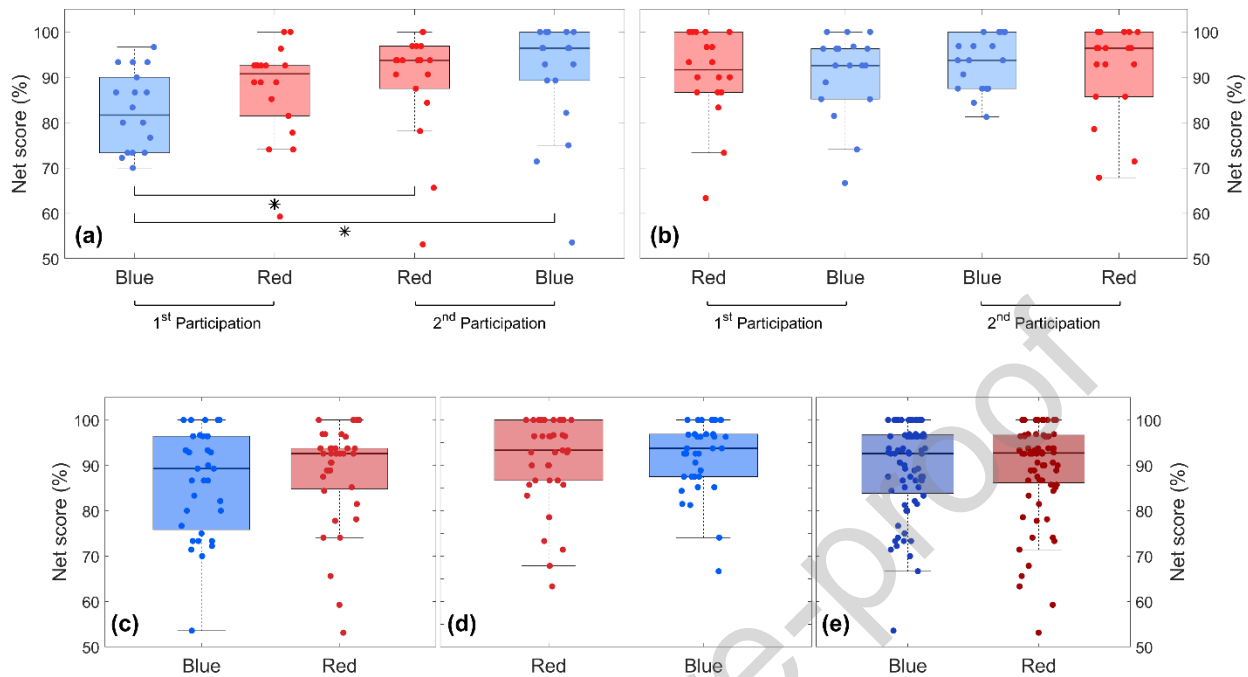


Figure 2. Boxplots showing the net scores of the 2-back tasks under CLE. The measurements were performed under two lighting conditions. During the 2-back task, subjects were exposed to the sequence of blue followed by red light or vice versa in a randomized crossover design. **(a)** Net scores of the subjects who experienced blue light followed by the red light during the first participation and red followed by the blue light during the second participation. **(b)** Net scores of the subjects who experienced red followed by the blue light on the first participation and blue followed by the red on the second participation. **(c)** Boxplots summarizing the net scores of the subjects who experienced blue light followed by the red light on the first participation and red light followed by the blue during the second participation (summary of **(a)** subplot). **(d)** Boxplots summarizing net scores of the subjects who experienced red light followed by the blue light on the first participation and blue light followed by the red during the second participation (summary of **(b)** subplot). **(e)** Overall summary of the 2-back net scores under blue or red light exposure, regardless of conditions and group of subjects (summary of **(c)** and **(d)** subplots). The asterisks indicate the significance level ($p < 0.01$, Wilcoxon signed-rank test).

The block-averaged (group level) changes in cerebral hemodynamics and systemic physiology elicited by 2-back tasks and CLE are shown in Figure 3 for blue-red and in Figure 4 for red-blue light exposure. For both conditions in the PFC, the typical patterns of cerebral activation, i.e., $[O_2Hb]$ (increase), $[HHb]$ (decrease), $[tHb]$ (increase), and StO_2 (increase) are found during the first CLE/2-back task. In the next phases including the first and second recovery and the second CLE/2-back task, noticeable changes in fNIRS signals are not observed. Both conditions elicited significant and almost similar changes in the fNIRS parameters of the PFC, whereas the red-blue condition induced more significant changes in $[O_2Hb]$ and $[tHb]$ compared to the blue-red condition in the VC. The sequence-dependent effects were also observed for StO_2 at the VC. While both colors caused relatively similar changes in the VC at the position of the first exposure, blue light caused higher

changes compared to red light in the second part of the exposure. In the recovery phases of both conditions, all hemodynamic and cerebral oxygenation changes did not return to the baseline levels. Regarding the systemic physiological parameters, similar patterns were observed for the parameters RR, HR, PRQ, MAP and HF in both CLE conditions. Irrespective of the sequence of the CLE, the changes in HR and MAP were higher in the first exposure than in the second exposure. After the first CLE/2-back task phase, almost insignificant changes are observed in HR and MAP for the other phases. For both conditions, RR increased during the CLE/2-back task phases, while RR changes in the recovery phases were at the same level of baseline. PRQ changes were different from RR changes in the CLE/2-back task phases (decrease during tasks, insignificant during recovery). SpO₂ changes were generally higher with blue light than with red light. The blue light caused significant stimulus-evoked changes at the onset of the CLE/2-back for both conditions. While red as the first color led to a slight increase in the red-blue condition, red as the second color caused no significant changes in the blue-red condition. The SpO₂ changes during recovery phases were insignificant. In addition, an interaction was found between the order of light exposure for the SCL data. While both colors caused the same changes in SCL in the first exposure position, red light caused greater changes compared to blue light in the second exposure. Color-dependent changes in the recovery phase of the LF component of HRV were striking. LF decreased during the first CLE/2-back stimulation and reached a plateau, and remained constantly at a low level during the first recovery and the second CLE/2-back stimulation. Finally, it is gradually elevated to the baseline level. Overall, significant differences between the two CLE conditions were observed for [O₂Hb]-VC during both 2-back tasks, for [tHb] and SCL during the second 2-back task and the second recovery phase, as well as for LF at the end of the first 2-back task and during the first recovery phase (green asterisks in Figure 3 & 4). Significant differences between the two 2-back tasks within one condition (blue-red or red-blue) were observed for fNIRS parameters in the PFC as well as for MAP, HR and LF for both conditions (red plus signs in Figure 3 & 4). Within condition, RR showed significant differences only during the red-blue CLE condition, whereas more significant differences between colors were found for the LF parameters in both the recovery and 2-back phases (red-blue condition).

The correlations between the Δ AUC values of systemic physiological parameters, fNIRS signals and 2-back task performance are shown in Figure 5 for blue and red light exposure. For both blue and red light exposure, the 2-back task performance was negatively correlated with [O₂Hb] in the PFC (red: $r = -0.37$, $p = 0.001$; blue: $r = -0.33$, $p = 0.004$) and positively correlated with the HF (red: $r = 0.35$, $p = 0.003$; blue: $r = 0.25$, $p = 0.04$), independent of CLE. Moreover, the negative correlations found between HF and HR (red: $r = -0.34$, $p = 0.003$; blue: $r = -0.35$, $p = 0.004$), and between [O₂Hb]

and [HHb] in the PFC (red: $r = -0.32$, $p = 0.007$; blue: $r = -0.57$, $p < 0.001$), as well as the positive correlations between [O₂Hb]-PFC and MAP (red: $r = 0.25$, $p = 0.04$; blue: $r = 0.28$, $p = 0.02$), and between [O₂Hb]-PFC and [O₂Hb]-VC (red: $r = 0.26$, $p = 0.03$; blue: $r = 0.28$, $p = 0.02$) were the same for blue and red CLE. However, differences were found between the two CLE conditions. For blue CLE, additional positive correlations were detected between [O₂Hb]-PFC and HR ($r = 0.26$, $p = 0.03$), and between [O₂Hb]-PFC and SCL ($r = 0.31$, $p = 0.01$) as well as between [HHb]-PFC and [HHb]-VC ($r = 0.25$, $p = 0.04$), besides that additional negative correlations were found between [HHb]-PFC and HR ($r = -0.34$, $p = 0.004$) and between [HHb]-PFC and MAP ($r = -0.25$, $p = 0.04$). For the red CLE, additional positive correlations were observed between SCL and [O₂Hb]-VC ($r = 0.27$, $p = 0.03$) and between P_{ET}CO₂ and PRQ ($r = 0.34$, $p = 0.005$), as well as an additional negative correlation between P_{ET}CO₂ and HR ($r = -0.31$, $p = 0.009$).

2-back Task at Blue-Red Light Exposure

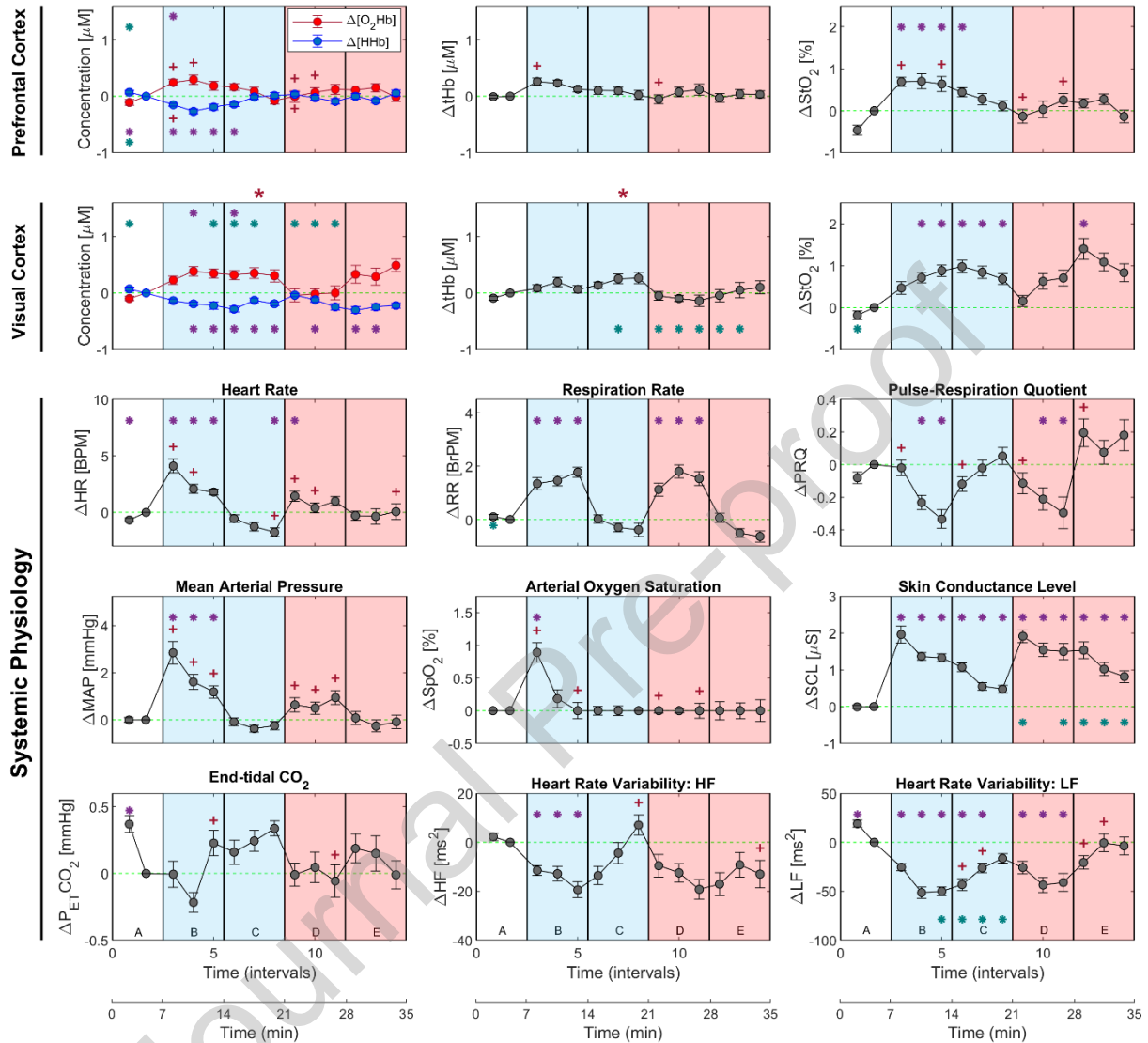


Figure 3. Block-averaged (group-level) changes in cerebral hemodynamics/oxygenation and systemic physiology (median \pm SEM) elicited by colored light exposure and 2-back tasks. The shaded areas represent the color light exposure and the subdivision of the different phases of the measurements. The different phases are labelled with the letters A, B, C, D and E. A stands for the baseline (No-light condition), B for the first 2-back task with blue light, C for the first recovery phase of blue light exposure, D for the second 2-back task with red light and E for the second recovery phase of red light exposure. The time series are divided into 14 periods of equal length and the value are all normalized to the last time period of the baseline. Purple asterisks indicate a significant change of the marked time point from baseline (proved by FDR-corrected p-values, $p < 0.05$, Wilcoxon signed-rank test). Green asterisks present a condition difference ("blue to red") of the marked time points ($p < 0.05$, Wilcoxon-Mann-Whitney test) and red plus signs present a color-difference (Blue vs. Red) of the marked time points within one condition ($p < 0.05$, Wilcoxon signed-rank test). Additionally, red asterisks represent a significant difference in a marked parameter between the two conditions ($p < 0.05$, cluster based permutation-test).

2-back Task at Red-Blue Light Exposure

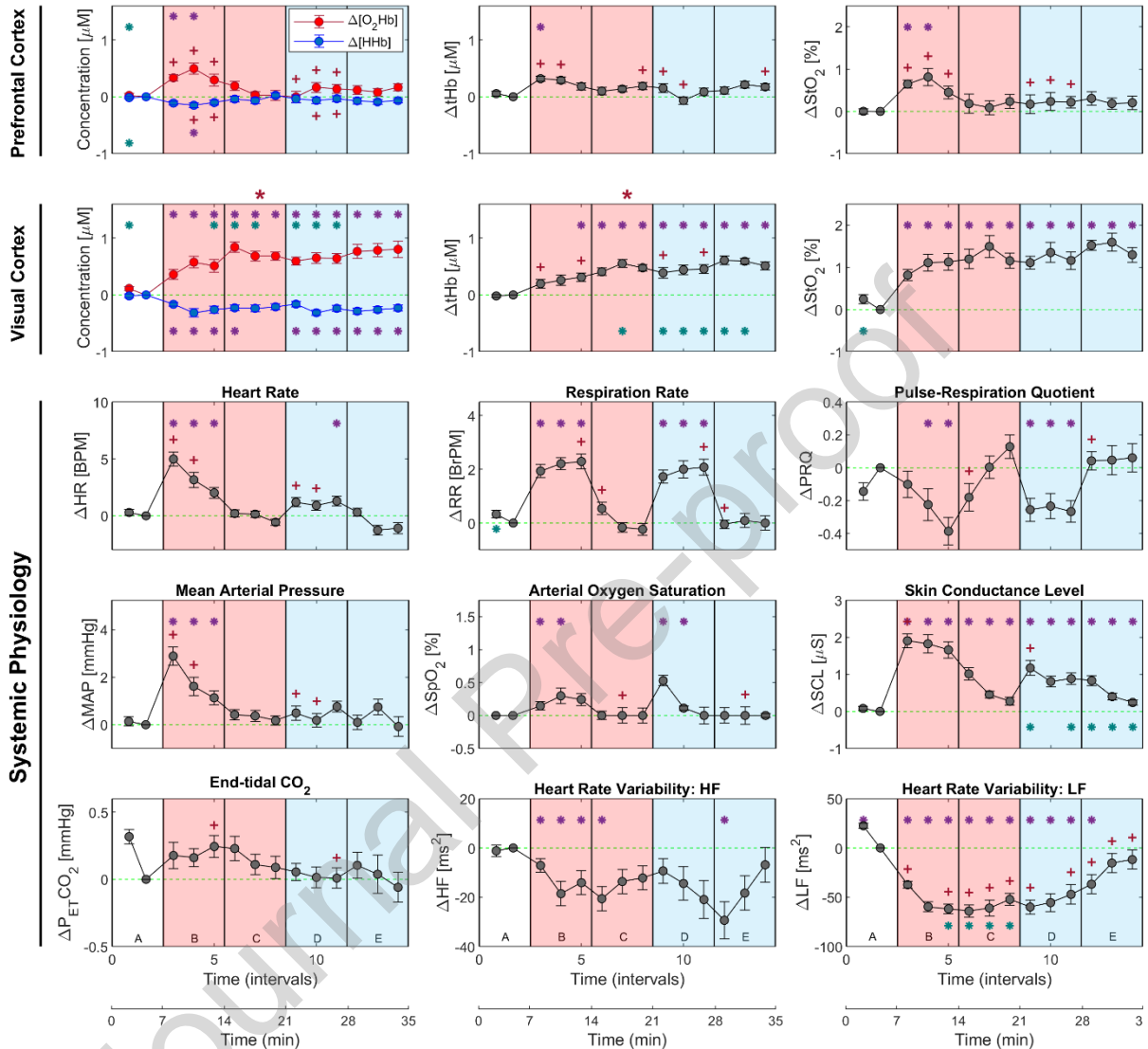


Figure 4. Block-averaged (group-level) changes in cerebral hemodynamics/oxygenation and systemic physiology (median \pm SEM) elicited by colored light exposure and 2-back tasks. The shaded areas represent the color light exposure and the subdivision of the different phases of the measurements. The different phases are labeled with the letters A, B, C, D and E. A stands for the baseline (No-light condition), B for the first 2-back task with red light, C for the first recovery phase of red light exposure, D for the second 2-back task with blue light and E for the second recovery phase of blue light exposure. The time series are divided into 14 periods of equal length and the values are all normalized to the last time period of the baseline. Purple asterisks indicate a significant change of the marked time point from baseline (proved by FDR-corrected p-values, $p < 0.05$, Wilcoxon signed-rank test). Green asterisks present a condition-difference ("blue to red" vs. "red to blue") of the marked time points ($p < 0.05$, Wilcoxon-Mann-Whitney test) and red plus signs present a color-difference (Blue vs. Red) of the marked time points within one condition ($p < 0.05$, Wilcoxon signed-rank test). Additionally, red asterisks represent a significant difference in a marked parameter between the two conditions ($p < 0.05$, cluster based permutation-test).

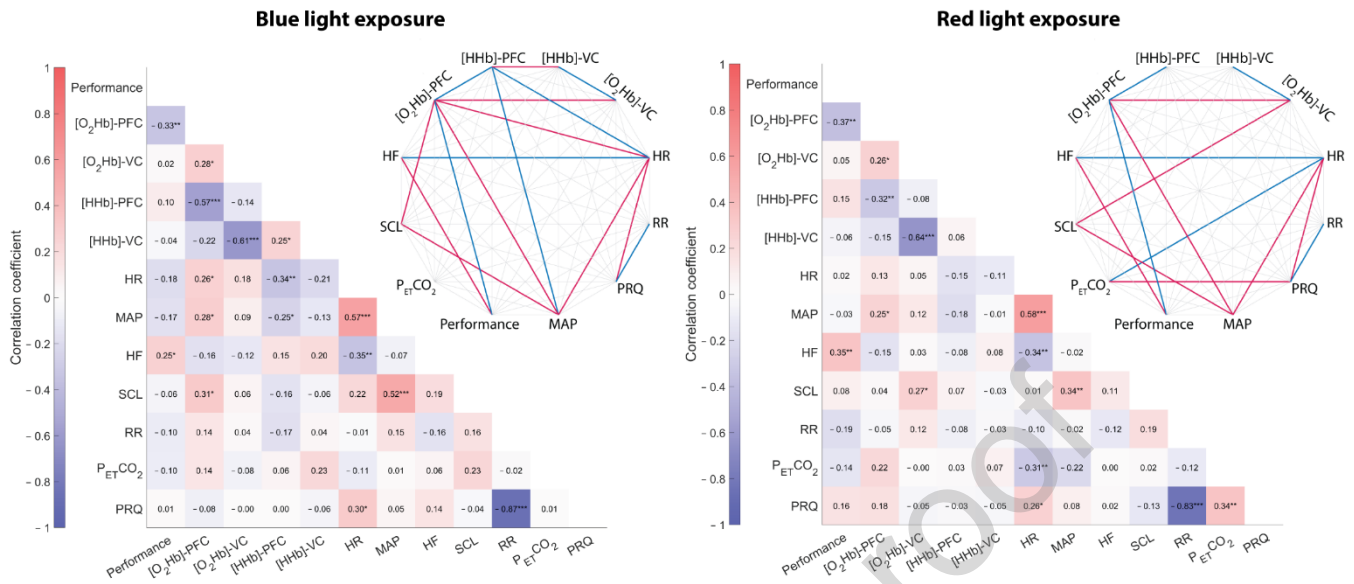


Figure 5. Correlation matrix of ΔAUC values of systemic physiological parameters, fNIRS signals and 2-back task performance obtained under blue and red light exposure. The significance of the correlation based on the p -values is indicated by the usage of asterisks next to the calculated correlation values (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$). The significant correlations between the parameters are also shown in the form of a polygon at the top right of the correlation matrix, with significant correlations between the parameters shown as colored lines ($p < 0.05$). Positive correlations are indicated by red, while blue indicates negative correlations between the parameters.

4. Discussion

Light (from natural and artificial sources) is not only necessary for vision, but also influences a wide range of behavioral and physiological functions, including learning and memory, sleep, mood and alertness [1,2,42–47]. In the current study, we aimed to determine how the performance of the 2-back task is affected by CLE with different colors, namely blue and red, as these are two of the three primary colors located at either end of the visible light spectrum in humans. We also used SPA-fNIRS to investigate how CLE, i.e., blue and red, and a 2-back task affect cerebral hemodynamics, oxygenation and systemic physiology.

One of the main findings of this study was that we found no significant difference in the subjects' performance between blue and red light, although performance was sometimes influenced by learning effects (Figure 2a), with one group of subjects being more successful at the second participation than at the first participation. There are three possible reasons for the non-significant difference between blue and red light exposure at the behavioral level. First, the subject's attention was mainly focused on performing the 2-back task and not on actively perceiving the CLE. This may have attenuated the effect of the specific colored light on performance [28]. Second, subjects were exposed to 120 lux illuminance, which is within the range of controlled lighting conditions and subject acceptance [48]. It was shown that artificial light applied at

intensities up to 2000 lux does not even elicit significant improvements in alertness, performance, and psychological well-being [49]. The use of higher illuminance conditions, (i.e., higher than the 120 lux used) is likely to have had a more pronounced and significant effect on the subjects' performance. Third, there are always a number of factors that can influence cognitive performance. These factors, which are in most cases difficult to assess and control during measurements, include individual personality, emotional state, color preference, social and lifestyle habits, culture and even socioeconomic status [27]. When using affective picture stimuli, one study found that negative affective valence negatively affected accuracy and reaction time in the n-back task [50]. Psychosocial stress with negative affect, including elevated salivary cortisol (as a marker of hypothalamus-pituitary-adrenal activity) and alpha-amylase (as a marker of sympathetic nervous system activity), as well as smoking abstinence, resulted in similar impairments in working memory [51,52]. The stimulating effects of CLE can have a positive, null or negative impact on task performance depending on individual differences (e.g. age, sleep-wake regulation), environmental conditions and psychological factors [53]. Although several studies have shown positive effects of illuminance on cognition by reducing reaction time, improving alertness and enhancing performance [53–60], our findings that performance is not significantly affected by lighting conditions are consistent with several studies [61–66].

Prior light exposure is known to influence subsequent responses to light [1]. However, although previous studies have reported the effects of prior light exposure on melatonin levels and phase shift [67,68], little is currently known about the role of prior light exposure on behavior and physiology in general. Therefore, one of the main reasons for using two color light sequences in our measurements was to investigate the effects of the light exposure sequence. These effects were clearly evident in some physiological parameters. The sequence-dependent effects were observed for StO_2 at the VC and for the SCL data. We showed that exposure to blue light evoked stronger overall responses in cerebral hemodynamics and oxygenation in the VC. The stronger response of the visual system to blue might be a marker for the central nervous system dopamine tone [69]. Moreover, light with short wavelengths is not captured in the straight guide, and most of the total power in these wavelengths leaks to the surrounding high-sensitivity rods, which are most sensitive to short wavelengths [70]. While such processes are known for the visual system [67], the sequence-dependent effects of illuminance on electrodermal activity were detected for the first time in this study. While both types of colored light elicited the same changes in SCL at the first light exposure, greater changes were observed for red light compared to blue light at the second light exposure. Our findings are in line with other research, where they showed an increase in electrodermal activity under red light [71,72]. Such differences between the two conditions could

also be due to the fact that the light history induced by the previous light exposure (blue light) causes a strong and long-lasting autonomic nervous system (ANS) response. In our previous studies, we also found an increase in the parasympathetic response after 15 min of blue light exposure [28]. SCL is considered an indicator of arousal and reflects the state and activity of the ANS [30,71]. It has been reported that red and blue light activate more sympathetic and parasympathetic ANS, respectively [73]. This is partially consistent with our results when we found color-dependent changes in the recovery phase of the LF component of the HRV, with LF changes being higher for red light exposure than for blue light. The LF component of HRV is generally associated with sympathetic nervous system activity related to the "fight or flight" response. Traditionally, the color red is often associated with increased arousal, attention and stimulation. It can be linked to alertness and heightened awareness, which aligns with aspects of the "fight or flight" response. This association with the color red is not only cultural, but also has some basis in physiological responses, as exposure to red light has been suggested to increase sympathetic nervous system activity more than blue light [74]. Consideration of the physiological effects of light history is critical because the invention of electric lighting and the ubiquity of electronic displays containing high levels of blue light, have tremendously altered human light exposure patterns [75]. These innovative lighting technologies may be detrimental to human health if the physiological effects of light are not considered.

In addition to the color-dependent changes explained above, a variety of physiological responses was elicited in the presence of stressors i.e., performing 2-back tasks e.g., an increase in SCL, MAP, HR, RR and a decrease in PRQ. In this study, regardless of the conditions, similar patterns were observed for the mentioned parameters in both conditions. This means that the impact of 2-back tasks is more prominent compared to CLE. In other words, the stimulating effect of CLE is low or even decreases when the brain is already involved in a challenging VFT task.

Based on the behavioral and fNIRS results, Figure 5 shows that performance in the 2-back task is negatively correlated with changes in $[O_2Hb]$ in the PFC, independent of the CLE. The PFC regions perform executive functions, such as higher-order cognitive functions, which are essential for planning and executing complex motor control actions [9,76]. The dorsomedial PFC, in conjunction with the dorsolateral PFC, has been found to be closely associated with monitoring behavioral performance and adapting behavior to external stimuli [77,78]. Yuan et al. suggested that weaker task-evoked changes in $[O_2Hb]$ mean that people are able to perform tasks easily [9]. There is also ample evidence that trained participants generally have lower brain activity in the PFC compared to novices and the control group [79–81]. Based on the literature discussed above, one interpretation of our results could be the greater changes in $[O_2Hb]$ were due to increased

task engagement, suggesting that those who did not perform well required more strenuous processing to cope with the workload. On the other hand, the better the subject's performance, the smaller the changes in cerebral oxygenation. The high-performing subjects were better able to complete the 2-back tasks with less effort. In addition, a positive correlation regardless of CLE was found between task performance and the HRV component, independent of CLE. In general, it is assumed that HF is low during mentally demanding tasks and high during periods of low exertion [82,83]. Lower HF may be attributed to poor cognitive performance as it reflects stress, anxiety or an excessive strain on cognitive resources, all of which may impair task-relevant processing [84], whereas good performance is associated with lower stress and consequently higher HF changes. Although some of the correlations found between different parameters were the identical in blue and red CLE, there were also differences between the two CLE conditions. For example, during red CLE, positive and negative correlations were observed between $P_{ET}CO_2$ and PRQ, and $P_{ET}CO_2$ and HR, respectively, whereas no such correlations were found during blue light exposure. The reasons for this observation are not yet clear but are probably caused by environmental conditions and other factors mentioned above, which influence not only cognitive performance but also cerebral and systemic physiological parameters. The dependence of systemic physiological parameters on several factors, including seasonal changes, temperature, mood, chronotype and time of day, has already been investigated in another of our studies [25].

This study has the following limitations: (1) Although the number of participants was calculated with a power analysis, a larger number of subjects may have shown more significant physiological or behavioral color-dependent responses. (2) The fNIRS measurement setup did not cover the entire head, and therefore the whole brain was not measured. Ideally, fNIRS measurements should probe the entire head in order to better capture hemodynamics and oxygenation changes, especially in areas related to working memory (e.g., inferior parietal cortex, middle temporal cortex and anterior cingulate cortex) [80]. (3) While red and blue are the primary and widely used colored lights in science and society, other colors should also be investigated with our experimental paradigm and SPA-fNIRS approach. (4) To eliminate the potential influence of the time of day, it would be worthwhile to (i) set the experimental time at a fixed time of day, i.e. in the morning hours, for all subjects and (ii) set the time of day with respect to the subjects' chronotypes. (5) Subjects were exposed to 120 lux illuminance, which is within the range of controlled lighting conditions and subject acceptance. Higher illuminance levels (e.g., > 500 lux) are likely to result in even more pronounced and distinct cerebral, systemic physiological and behavioral responses.

5. Conclusions

Investigating the interaction between colored light and human physiology is crucial because CLE is common in everyday life and these effects have hardly been studied so far. This is the first study to investigate how CLE and the 2-back task affect subjects' performance, cerebral hemodynamics, oxygenation and systemic physiology. Although no significant difference was found in subjects' performance in the 2-back task between red and blue light exposure, the sequence-dependent effects in changes in StO_2 were observed in the VC and for the SCL data. The findings of this study are useful to better understand the influences of prior light history in humans. Light history affects the subsequent response to light [1]. Therefore, our results merit future studies to investigate the effects of light histories that humans normally experience more frequently, e.g. in everyday life. The sequence effect of light exposures should be considered in experimental and clinical settings. The application of these results could also be crucial for the treatment of light-induced sleep disturbances during shift work and could therefore be considered in treatment plans [67]. The clinical implication of such findings may enable the optimization of light therapy in the treatment of circadian phase misalignment. Our results could be applied to light therapy, which is considered to be a safe, effective and inexpensive modality for various clinical applications, such as improving sleep and cognition, which are important for daily human activities. Some therapies, such as chromotherapy or colored light therapy, propose that exposure to specific colors can have therapeutic effects on physical and mental health. Choosing the optimal sequence and timing of exposure to different colors is an essential part of these practices. Finally, knowledge of the cerebral and physiological effects of colored light can help determine when users should be more relaxed, for example, or when their attention should be engaged in educational applications.

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Informed Consent Statement: Written informed consent was obtained prior to the measurements.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Hamoon Zohdi: Conceptualization, methodology, data analysis, visualization, writing—original draft preparation; **Josefa Märki:** Experiments, data analysis, writing—original draft preparation;

Felix Scholkmann: Conceptualization, methodology, writing—review and editing, supervision;

Ursula Wolf: Conceptualization and methodology, resources and funding acquisition, writing—review and editing, supervision