



Transcranial near-infrared photobiomodulation could modulate brain electrophysiological features and attentional performance in healthy young adults

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Abstract

The aim of the present study was to investigate the electrophysiological effects of the photobiomodulation (PBM) by the quantitative electroencephalography (qEEG) as a diagnostic method. The neurotherapeutic potential of transcranial PBM has been recently investigated in preclinical and clinical studies. According to the PBM mechanisms of action on increasing the cerebral blood flow and the neuronal firing, a change may occur in cortical electrical activity after transcranial PBM that could be revealed in qEEG. A total of 30 participants (15 males and 15 females) were included in this experimental study in a convenience sampling method. A 19-channel EEG was obtained from subjects, before and after receiving sham or real 850-nm PBM by light emitting diode (LED) array on the right prefrontal cortex (PFC). An attentional task also was completed by the participant before and after the irradiation. Results presented that the effect of PBM on the reaction time was significant ($p = 0.001$) in favor of the real-treatment group ($p < 0.05$). For the absolute power, repeated-measures ANOVA showed a significant interaction of group \times time \times frequency ($p = 0.04$). In the real-treatment group, absolute power of delta band was significantly reduced in all electrodes ($p < 0.05$). Also, a similar significant interaction of group \times time \times frequency was seen for relative power ($p = 0.04$). Post-hoc analysis showed a significant decrease in delta band after PBM in the real treatment group ($p < 0.05$). The study presented that light irradiation with 850-nm LED source on right PFC could change brain electrical activity and has beneficial effects on attentional performance.

Keywords Transcranial · Near-infrared · Photobiomodulation · Attentional performance · Quantitative electroencephalogram

Introduction

Transcranial photobiomodulation (PBM) is a novel and safe neuromodulatory procedure in which red and near-infrared (NIR) light is irradiated on the intended area of the scalp

[1]. Light at this spectrum could partially penetrate into the brain [2] and potentially induce many neurobiological changes by its non-thermal effects [3, 4]. Coherent light produced by laser devices is commonly photon sources for PBM therapy [5, 6]. However, recently, there has been a growing interest in using relatively inexpensive light-emitting diode (LED) devices which so far has been found to be totally devoid of any side effects [7–9]. Cytochrome c oxidase (CCO), the terminal enzyme in the mitochondrial respiratory chain, is considered as the main photo acceptor for red/NIR light (600–900 nm). This has a light absorption peak at 850 nm [10, 11]. Light activation of CCO leads to enhancement of the cellular metabolic energy, ATP production [5, 12], and improvement of focal cerebral blood flow (CBF) due to releasing nitric oxide [4, 13]. In addition, NIR light could prevent neuronal cells from death by regulating the gene expression [6, 14].

Studies on physiological mechanisms of the PBM support its applicability in neurological and psychological conditions,

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as an effective neurorehabilitation modality [6, 15, 16]. Transcranial PBM has exciting potential to improve cognitive performance [7, 8, 17]. In this respect, it has been shown that NIR light irradiation to the forehead promotes sustained attention, memory, executive function, and some other higher level cognitive abilities [8, 18, 19]. According to the literature, the effectiveness of the transcranial PBM for cognitive rehabilitation is mostly based on cerebral metabolic improvement and the increase in regional CBF [4, 18–20].

Considering the key role of the quantitative electroencephalogram (qEEG) in diagnostic and therapeutic procedures such as neuro-feedback, understanding the effect of transcranial PBM on qEEG seems to be useful for neuroscience researches. A great interaction was reported between changes in cerebral perfusion, blood flow, and the alteration of brain electrical activity in healthy subjects [21, 22]. So, the PBM therapy could change brain electrical activity following the increase of regional CBF and perfusion. Change in EEG oscillation also could occur after PBM due to the activation of calcium channels in the brain cellular membrane [22, 23].

A few electrophysiological studies were inspected the impact of NIR light on features of the brain electrical activity and the neuronal functions. Grover et al. reported that PBM therapy could change EEG amplitude in a subgroup of participants who had a lower EEG amplitude. Some measures of the brain electrical activity such as reaction time and amplitude of the event-related response have been changed after near light therapy in his study [22]. Using laser, Wang et al. reported increased alpha power after light irradiation with 1064 nm and 1452 J parameters [24]. Vargas et al. also investigated the effect of prefrontal 1064 nm and 250 mW/cm² laser irradiation on qEEG of older adults with neurocognitive complain. They presented that this dosage of laser light could change brain alpha, beta, and gamma power associated with increasing the brain oxygenation [25]. However, as we know, there was no study on the possible effects of the near-infrared LED irradiation on brain frequency bands to understand its biophysical effects [5]. Our previous study revealed the effectiveness of the 850-nm LED with 60 J energy density on cognitive performance [8]. Therefore, it seems that in-depth understanding of the transcranial LED mechanism to provide more effective clinical applications is viable by qEEG studies. This study aimed to investigate the possible electrophysiological changes of the brain associated with transcranial LED-PBM by using qEEG in healthy people.

Materials and methods

Experimental design

This study was conducted at the University of Tabriz by a convenience sampling method. A total of 30 healthy young adult

participants (15 females; age range 18–25 years and 15 males; age range 19–25 years) were enrolled in this research. According to the inclusion and exclusion criteria, right-handed and white skin subjects with no history of neurological or psychological disorder were included in this study [8, 26]. Each participant completed an information questionnaire including biographical and medical details (sex, name, age, telephone number, dominant hand, and history of the neurological or psychological disease). The sample size was calculated based on the formula for comparison of the groups means using software package G*Power (v.3.1.9.2., Kiel, Germany), according to a previous study [8, 27]. Alpha and Beta were considered as 0.05 and 0.2, respectively. Simple random allocation strategy was used for assigning the participants in the real or sham-control group. Figure 1 presents a flowchart of the study design.

Ethical considerations

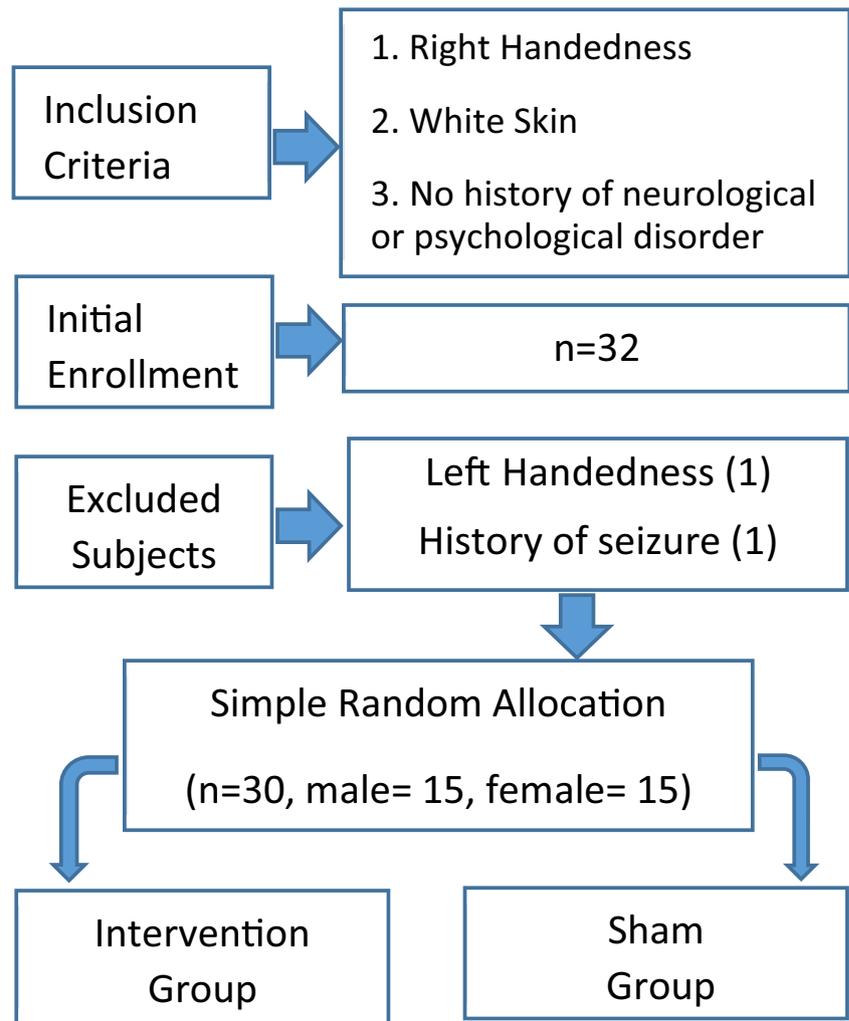
The study was approved by the Regional Ethical Committee of Tabriz University of Medical Sciences (No: IR.TBZMED.RCE.1395.687). Upon arrival, participants completed the informed consent form including the information about the safety of the device and procedures. Participants were reassured that they could leave the study at any time. For the sake of confidentiality, the personal data were coded.

EEG recording

All EEG signal was acquired by the NeuroScan system (Compumedics Neuroscan, USA) and amplifier attached to an elastic cap (Electro-Cap, USA). Ten-minute eyes-open resting EEG was recorded from electrodes Fp1, Fp2, F3, Fz, F4, F7, F8, C3, Cz, C4, P3, Pz, P4, T5, T6, O1, O2, A1, and A2 in the linked-ears referential montage. The sampling rate was 256 Hz and a band-pass filter was applied between 0.5 and 30 Hz on the signal. Impedances of all electrodes were kept below 5 k Ω .

Attentional task

The attentional task was completed just after EEG recordings in pre- and post-procedures. Parametric Go/No-Go task, as a valid and strong reliable test, was applied to measure sustained attention. Black color letters (a, b, j, l, r, s, t, x, y, and z) with Times New Roman font (size 40) were presented for 500 ms at the center of the screen by the DMDX software (version 5.1.3.4). The participants were asked to respond to the task by pressing the space bar immediately after presentation of the X, Y, and Z letters [8]. The reaction time of the response (RTT) was calculated by averaging the response time to all correct trials which present the processing speed of the participants in multiple target tasks [8]. Schematic view of the parametric Go/No-Go task is represented in Fig. 2.

Fig. 1 Flowchart of the study design

Transcranial PBM procedure

According to our previous work [8], the light source was a super-luminous multi-LED array (Iranbargh, Tehran, Iran) containing 20 NIR LEDs with a peak wavelength of 850 nm, which was calibrated by the Lumix3 internal calibrator (Lumix3, Italy). The total power was 400 mW with a 1.4-cm² irradiation area; thus, the applied power density was 285 mW/cm². Participants were given a 2.5-min single-session irradiation transcranially on the Fp2 region, according to the 10–20 international EEG system [28], which is responsible for high-level cognitive ability such as vigilance [20]. The device delivered a total dose (energy density) of 60 J/cm² to the surface of the scalp. The members of the sham group were treated the same as the real intervention group, except that they received only a 5-s light irradiation for each 1-min of the treatment (Fig. 3a, b).

It is notable that before transcranial PBM procedure, the Fp2 region was prepared for the light irradiation by folding the EEG cap from the forehead and cleaning the skin from additional ingredients such as the EEG conductive gel. At the end

of light irradiation, the EEG cap was replaced back onto the forehead. After rechecking the impedance and signal quality, the post-PBM signal was recorded.

Data processing

Before EEG signal processing, artifacts were inspected and rejected visually. At least 36 to 48 artifact-free EEG epochs (2.5 s) were selected throughout the raw EEG for analysis. The selected epochs were then transformed into the frequency domain using a fast Fourier transformation algorithm (FFT, 512-point). Absolute and relative powers were calculated for all channels using Neuroguide software version 2.9.7 (Applied NeuroScience, Inc.) for the following frequency bands: delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), and beta (12–30 Hz). Absolute power (AP) is defined as the square of voltage in each frequency band of EEG signal and the relative power (RP) as the percentage of power in any frequency band to the total EEG power [29]. To designate the brain regions of interest, the electrodes were averaged in eight groups: left frontal, right frontal, left temporal, right temporal,

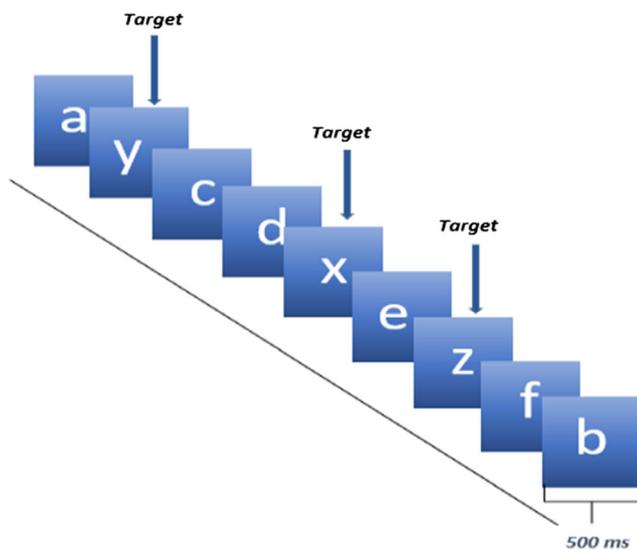


Fig. 2 Parametric Go/No-Go task for measuring sustained attention. The participants were asked to respond to the target stimulus (x, y, and z letters) by pressing the space bar. (Stimulus duration, 500 ms and inter-stimulus interval, 0 ms). The reaction time of the response (RTT) is calculated by averaging the response time to correct trials (targets). This variable presents the processing speed and sustained attention

left central, right central left parietal, and right parietal which is represented in Table 1.

To measure the participants' attentional performance, the RTTs were extracted from the output file provided by DMDX software. It is notable that the RTT is faster than 200 ms or fast impulsive response, and the RTT is longer than 1000 ms was assumed as invalid data and was removed from further analysis.

Statistical analysis

The K-S test was used to determine the normality of the data. To analyze the data, the repeated-measures

ANOVA (rANOVA) was used. Due to the violation of the sphericity assumption, a Greenhouse-Geisser correction was applied. Group of assignment (real/sham) and sex (male/female) were considered as between-subjects factors and time (pre-PBM/post-PBM) as a within-subject factor. Interaction of group \times time was assumed to indicate the effect of PBM.

Results

Demographic/clinical characteristics of the participants are presented in Table 2.

Behavioral data

Reaction times before and after treatment were normally distributed ($p > 0.1$) in both treatment groups (real and sham). The effect of PBM on the reaction time was analyzed by rANOVA. The test showed a significant group \times time interaction [$F(1, 29) = 13.45, p = 0.001, \eta^2 = 0.34$] (Fig. 4). Post-hoc comparison showed a significant decrease in the reaction time only in the real-PBM group ($p < 0.05$).

The main effect of group was not statistically significant [$F(1, 29) = 0.17, p = 0.68$], indicating a successful random assignment of participants. Also, there was no significant difference in reaction time between men and women [$F(1, 29) = 0.18, p = 0.61$].

There was no significant interaction between sex and time [$F(1, 29) = 0.51, p = 0.47$]. In addition, the interaction of group \times sex \times time was not statistically significant [$F(1, 29) = 0.41, p = 0.52$].

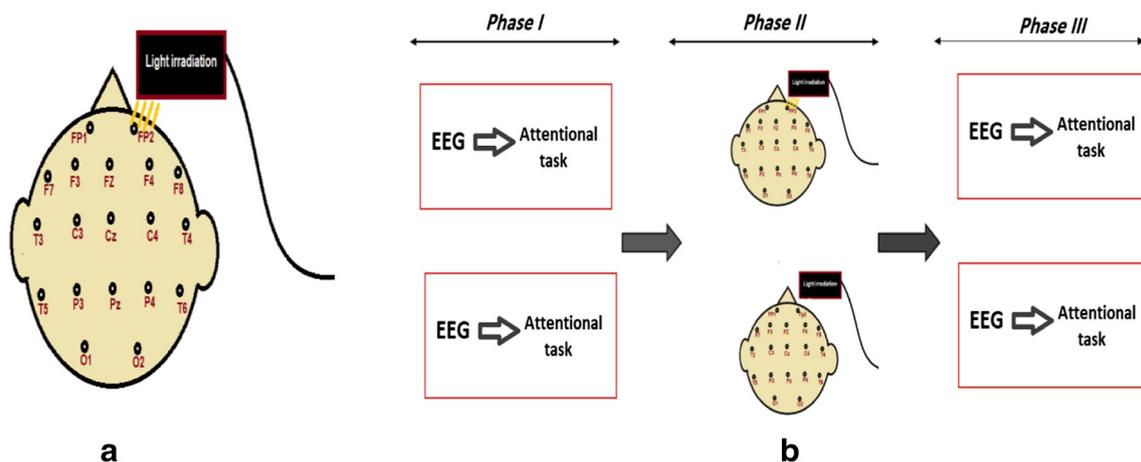


Fig. 3 a The light source (super-luminous multi-LED array with peak wavelength of 850 nm, the total power 400 mW with 1.4 cm² irradiation area, and power density 285 mW/cm²), irradiated transcranially on the Fp2. b The 60-J/cm² light irradiated upon the surface of the scalp in the

intervention group. The sham group received only a 5-s light irradiation for each 1-min. The attentional task was completed just after EEG recordings in pre and post procedures

Table 1 The averaged electrodes for representing the brain regions of interest

| Frontal | | Temporal | | Central | | Parietal | |
|---------------------|---------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Left | Right | Left | Right | Left | Right | Left | Right |
| Fp1, F7, F3, and Fz | Fp2, F8, F4, and Fz | T3 and T5 | T4 and T6 | C3 and Cz | C4 and Cz | P3 and Pz | P4 and Pz |

The qEEG data

Absolute power

As the distribution of AP data in both groups for eight regions was not normal, the natural-log (Ln) transformation was done and finally the normality was confirmed by Kolmogorov-Smirnov test ($p > 0.05$). A repeated-measures ANOVA was applied. The group with two levels (sham, real) was included as between-subject factor and the time (two levels: pre-PBM, post-PBM), the regions (four levels: frontal, temporal, central, parietal), the frequency (four levels: delta, theta, alpha, beta), and the hemisphere (two levels: left, right) were included as within-subject factors.

Analysis showed that interaction of group \times time \times frequency was statistically significant ($F = 3.23$, $df = 2.26$, $p = 0.04$, $\eta^2 = 0.10$). No interaction of the region or hemisphere, with group and time was seen (p values > 0.05). Post-hoc analysis showed a significant difference only in delta band (Fig. 5a). In other words, delta decrement was observed after real-PBM in all regions ($p = 0.02$). Also, post-hoc analysis showed a significant increase in the absolute power of the delta band in all regions after sham-PBM ($p = 0.04$).

Relative power

The data for RP was distributed normally according to Kolmogorov-Smirnov test in all regions for both groups (p values > 0.05). Similar to AP, for RP, repeated measures-ANOVA showed a significant interaction between the group, time, and frequency ($F = 3.92$, $df = 1.50$, $p = 0.04$, $\eta^2 = 0.13$). Post-hoc analysis showed a significant decrease in delta band

Table 2 Demographic/clinical characteristics of the participants

| Variables | Real | Sham | p values |
|---|--------------|--------------|-------------|
| Age ^a | 21 \pm 2 | 21 \pm 2 | $p = 1.0^c$ |
| Gender ^b | 53 | 53 | $p = 0.6^d$ |
| Educational level ^a | 13 \pm 0.5 | 13 \pm 0.1 | $p = 0.4^c$ |
| History of psychological/neurological disorders | None | None | $p = 1.0^d$ |

^a Years expressed as mean \pm SD (standard deviation)

^b Frequency of females expressed as no. (%)

^c Independent t test

^d Chi-square test

after real-PBM (Fig. 5b). As no interaction of the region and the hemisphere was seen by the time and group, decrement of the relative power of delta was inferred to occur in all regions.

Discussion

In this study, we showed for the first time that transcranial NIR PBM with LED source to the right prefrontal region of the head caused a significant change in brain electrophysiological features which was a reduction of the whole cortical delta. We also observed an improvement in the reaction time in the attentional task after treatment that indicated improvement of vigilance in participants after PBM. In a similar study, Wang et al. inspected the effectiveness of the laser PBM on qEEG. They found alpha power increment after light irradiation with 1064-nm laser stimulation with 1452 J dose [24]. In Vargas et al., they reported an increase in alpha, beta, and gamma bands after 1870-J laser irradiation in older adults with memory complaint. Some methodological limitations are seen in their study including small sample size ($n = 6$ for EEG study) and lack of the sham group. This could threaten as the validity of their EEG findings [25]. Modulation of the different frequency bands in these two studies could be related to differences in light modalities and PBM parameters. Laser source produces a monochromatic and coherent wavelength which could penetrate to the tissue with great amount of energy and constant beam width. However, the LED device products quasi-monochromatic and less collimated beam than laser which is not coherent [4, 5]. So, the photobiological effects

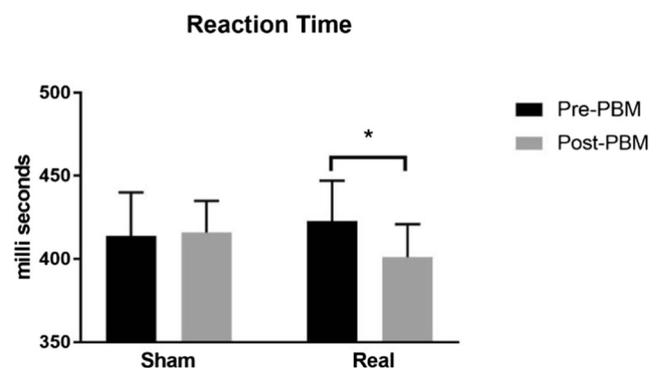
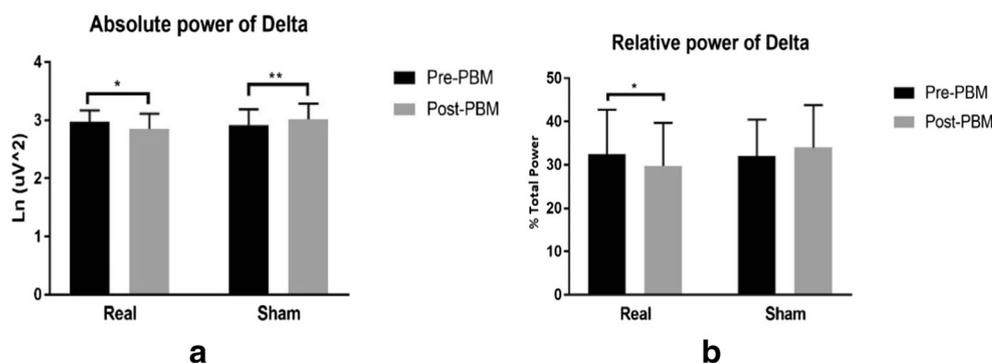


Fig. 4 The mean reaction times before and after transcranial PBM (photobiomodulation) in real- and sham-treatment groups. *Significant at $p < 0.05$

Fig.5 a Absolute power of delta band before and after transcranial PBM in real- and sham-treatment groups (represented in Ln). **p* value = 0.02, ***p* value = 0.04. b Relative power of delta band before and after transcranial PBM in real- and sham-treatment groups. **p* value < 0.01



of these sources is not essentially similar and could arise different neuronal activity pattern.

Decrease in the delta power simultaneously with the promotion of the attentional performance after light therapy in this study is in agreement with the previous findings, which investigated the sustained attention and vigilance by the qEEG. They reported the lower amount of the slow waves in high vigilance subjects compared to the low vigilance one. Also, a drowsy state or looseness of vigilance could be accompanied by a greater oscillation of the brain slow waves and a decrease in high-frequency powers in both rest and task conditions [30, 31].

In accordance with our findings, previous brain PBM studies have reported an enhancement of cognitive abilities in human cases and animal models [10, 18, 32]. In this regard, it has been shown that transcranial PBM could promote attentional performance [20, 32], executive function [19], and memory [33–35] by increasing neuronal metabolic energy levels and an increase in the regional CBF [8, 11].

Improvement of the high cognitive performance by the transcranial PBM serves as the NIR mechanism of action on brain metabolic capacity and an increase in the CBF [18]. The decrease in the delta power after light therapy is agreeing with these finding, as the delta oscillation has an inverse correlation with the amount of the focal oxygen saturation and the metabolism of the PFC [36]. An inverse condition also has been reported in brain hypoxic conditions and reduction of the focal blood flow in ischemic stroke and hypoxia, which are associated with an increase in slow waves generation [37, 38]. It seems that increased oxygen production and improvement of the brain CBF after light therapy affect the basic cortical generators of the delta band which is maximally located in the medial prefrontal cortex, anterior cingulate, and the orbitofrontal cortex [39].

The decrease in the slow waves in rest condition in is a goal of the treatment in some neuro-rehabilitation interventions. In fact, higher delta activation in awake state is associated with the almost any pathological conditions and cognitive impairment, the brain tissue damage and developmental or organic disorders [36]. The relative increase in the delta power is associated with an attention deficit and hyperactivity disorder,

dyslexia, Down, and the fetal alcohol syndromes [36]. Excessive delta power also was seen in adults with anxiety [40], depression [41], and Alzheimer's disease [42]. In addition, the anhedonia as a non-clinical predictive factor of the depression and schizophrenia is associated with enhancement of the delta wave density in the anterior cingulate cortex [43]. According to these findings, it is inferred that the promotion of the cognitive performance in this study could be relevant to the modulation of the delta power after transcranial BPM.

Despite the declined delta power in the real light therapy group, a significant increase in the delta absolute power was seen in the sham group (Fig. 5a, b). EEG studies have reported growth of the slow wave oscillation in central fatigue conditions, suggesting the tendency of the subject to rest and sleep [44, 45]. Accordingly, it seems that the increase in the delta wave in the sham group may be due to the exhaustion of the data collection process which has been reversed in the intervention group by the beneficial effects of the light irradiation. It has been evidenced that the increased brain mitochondrial bioenergetics is responsible for increasing resistance to fatigue by reduction of the centrally mediated factors. It seems that PBM could lessen the neuronal fatigue by its specific role in enhancing the mitochondrial function and the brain metabolism [10, 11].

There were some limitations in this study. We had no access to direct measurement of the regional CBF or metabolic changes after PBM. Therefore, this study is unable to reveal the exact neural basis of EEG changes after light irradiation. We also were unaware of the amount of absorbed light energy by the cortex, which could be affected by skin color, skull thickness, and bone density. Estimation of the absorbed energy by the cortex is suggested in future researches.

Conclusion

Briefly, our study suggests that transcranial PBM with 850-nm LED source on the right prefrontal cortex could change the brainwaves and have a beneficial effect on cognitive performance. However, additional researches on PBM are necessary to discover its neural basis, long-term effects, and dosage in healthy and clinical population.

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Compliance with ethical standards

The study was approved by the Regional Ethical Committee of Tabriz University of Medical Sciences and all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments.

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants The study was approved by the Regional Ethical Committee of Tabriz University of Medical Sciences (No: IR.TBZMED.RCE.1395.687).

Informed consent Informed consent was obtained from all participants included in the study.

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