Significant Increases in EEG Anterior-Posterior Alpha and Beta Powers By Transcranial Photobiomodulation (tPBM) in Healthy Humans With Exclusion of Thermal Effects

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Abstract

Transcranial photobiomodulation (tPBM) of the prefrontal cortex can improve human cognition and increase electroencephalogram (EEG) alpha and beta powers, but it was unclear whether tPBM-induced heat would influence EEG oscillation powers. This study aimed to prove that tPBM-induced increases in anterior-posterior EEG powers at alpha and beta bands would be significant after removal of tPBM-associated thermal effects. We performed both sham-controlled tPBM and sham-controlled thermal stimulation (thermo_stim) experiments under the eyes-closed resting state with concurrent recordings of 64-channel EEG before, during, and after 8-min tPBM at 1064-nm wavelength and thermo_stim with temperature from 33 to 41 °C, respectively, from healthy humans (n=46 for tPBM; n=14 for thermo_stim). Sham-subtracted topographies of EEG powers at five frequency bands were averaged at the group level during and post both stimulations. Two-sample t-tests with FDR correction and effect size were calculated for comparing tPBM and thermal effects at all five frequency bands. Right-frontal tPBM induced significant increases in EEG anterior-posterior alpha and beta powers under the eyes-closed conditions, consistent with the results previously reported in the eyes-open tPBM experiments. In contrast, right-frontal thermal stimulation under the eyes-closed resting state resulted in opposite effects on EEG power patterns with respect to those by tPBM. tPBM-induced enhancement in alpha and beta oscillations occurred during the 8-min intervention after exclusion of thermal effects. The ability of tPBM to synchronize alpha and beta oscillations in the anterior-posterior regions may be linked to the enhancement of frontoparietal network and the improvement of human cognition.

Keywords:

transcranial photobiomodulation, tPBM, EEG, thermal stimulation, brain oscillations.

1. Introduction

Photobiomodulation (PBM), also known as low-level laser therapy (LLLT) in clinical applications, utilizes red to near-infrared (NIR) light to stimulate mitochondrial respiration in a wide range of cells and tissues in the human body¹⁻⁴. Transcranial photobiomodulation (tPBM) is a type of PBM that delivers NIR light/laser to the human brain, which has shown promising outcomes in treating psychiatric and neurological disorders⁵, such as depression and anxiety⁶, and traumatic brain injuries^{7,8}. Recent studies have reported that tPBM with a 1064-nm laser can enhance human cognitive performance on a variety of cognitive tasks using sham-controlled experiments⁹⁻¹³. Furthermore, we have recently demonstrated that 1064-nm tPBM enabled significant upregulation in concentrations of hemoglobin oxygenation ([HbO]) and oxidized cytochrome-c-oxidase ([CCO]) during and after tPBM on the human right forehead with high reproducibility and robustness¹⁴⁻¹⁶. These findings supported and validated the hypothesized mechanism of action that tPBM can photo-oxidize CCO, the key mitochondrial enzyme for cellular oxygen metabolism, to boost the metabolic activities of cells¹⁷, especially neurons^{1,18}. In addition, we investigated the thermal impact of tPBM on measured alterations in [HbO] and [CCO], confirming that the heat-induced warm sensation on the forehead would not give rise to the same increases in [HbO] and [CCO] as those seen by tPBM¹⁹.

There has been much less understanding and observation of electrophysiological responses to tPBM in the human brain. Our recent results revealed that tPBM is effective in enhancing the EEG powers of large-scale alpha and beta oscillations in the human brain during eyes-opened resting state, measured by 64-channel scalp EEG from healthy human subjects^{20,21}. Similar observations on EEG responses to tPBM were reported by other groups while using different experimental

protocols²²⁻²⁴. All these studies consistently indicated that tPBM can also modulate neuronal or electrophysiological synchronization and connectivity in the human brain.

It is expected and often experienced that a sizeable optical beam used for tPBM may produce a warm sensation on the subject's forehead. Since the scalp EEG signal is sensitive to ambient temperature or thermal stimulation of the subject's head²⁵, the warmth created by the light/laser illumination during tPBM can potentially affect or contaminate the net EEG signal induced only by tPBM. Thus, an essential reservation or question on the action mechanism of tPBM would be whether the improvement of behavioral performances and EEG power alteration might result from, at least partially, thermal effects caused by light or laser illumination delivered on the subject's forehead. Furthermore, it is known that there exist distinct temporal and spatial patterns in human EEG signals between eyes-open and eyes-closed resting conditions²⁶. EEG signals are more vulnerable and sensitive to eye-blinks and drowsiness in the eyes-open resting state than in the eyes-closed resting state. This raised another question on whether tPBM-induced power changes of brain oscillation would be different between eyes-open and eyes-closed human resting state.

To address the two questions given above, we hypothesized that tPBM-induced increases in anterior-posterior EEG powers at alpha and beta bands remained statistically significant during the eyes-closed resting state, similar to those during the eyes-open resting state, after removal of tPBM-associated thermal effects. Using the same experimental protocol as our previous studies [3, 7, 11, 15], we performed both sham-controlled tPBM and sham-controlled thermal stimulation (thermo_stim) experiments. Specifically, we recorded 64-channel EEG before, during, and after 8-min tPBM and thermo_stim, respectively, from healthy human subjects (n=46 for tPBM; n=14 for thermo_stim) under the eyes-closed resting state. Alterations in EEG power at five frequency bands (delta, theta, alpha, beta, and gamma) and topographical patterns induced by each of tPBM

and thermo_stim were then computed and compared. All these observations allowed us to prove our hypothesis, as progressively presented in the following sub-sections.

2. Materials and Methods

2.1. Participants

For the sham-controlled tPBM experiment, 49 healthy human subjects (30 males, 19 females; 26 ± 8.8 years of age) were enrolled from the local community of the University of Texas at Arlington. However, 3 subjects were removed from the dataset due to self-reported/observed tiredness and/or sleepiness during the experiments. For the sham-controlled thermo_stim experiment, another group of 14 human subjects (8 males, and 6 females; 29 ± 8.8 years of age) were recruited from the same community. No substantial age difference between the two gender groups (with a two-tailed t-test, $p > 0.1$) was observed. Exclusion criteria of participants: (1) previous diagnosis with a psychiatric disorder, (2) history of neurological disease, (3) history of severe brain injury, (4) history of violent behavior, (5) prior institutionalization or imprisonment, (6) current intake of any psychotropic medicine or drug, (7) history of smoking, (8) excessive alcohol consumption, (9) pregnancy, and (10) previous diagnosis with diabetes as required by the manufacturer of the laser (Cell Gen Therapeutics LLC, Dallas, Texas). All the participants were told to avoid any caffeine beverages 2–3 hours before each experiment. All experimental procedures were approved by the Institutional Review Board of the University of Texas at Arlington; all methods were performed in accordance with the relevant guidelines and regulations. Informed consent was obtained from each participant prior to all experiments.

2.2. Experimental Setup

As shown in Fig. 1(a), a continuous-wave (CW) 1064-nm laser (Model CG-5000 Laser, Cell Gen Therapeutics LLC, Dallas, TX, USA), cleared by the Food and Drug Administration (FDA), was

utilized to conduct tPBM and the corresponding sham experiment in this study. This was the same device used in our previous studies^{11,19,20,27}. This laser was able to deliver a collimated beam in a diameter of 4.2 cm. We conducted tPBM with a total power of 3.5 W, which led to a power density of ~ 0.25 W/cm² and a total energy dose of 1680 J over 8 min of tPBM ($3.5 \text{ W} \times 480 \text{ s} = 1680 \text{ J}$) on the right forehead, as indicated in Fig. 1(b). For the sham experiment, the laser device was on but set to be 0.1 W during the 8-min stimulation time. In addition, a black colored cap was used to block the laser aperture. As a result, the power density under the sham stimulation was further confirmed to be 0 W/cm² by a sensitive power meter (Model 834-R, Newport Corp., Andover, MA, USA) to ensure the complete impediment of laser light. The participants were instructed to maintain their eyes closed with a minimal level of motion during the experiment. Subjects were also directed to give minor hand gestures in response to the experiment operator for verifying that they were not asleep during the experiment. At the end of the experiment, each participant was asked to confirm that he/she was awake without much drowsiness and sleepiness during the entire experimental period.

A Biosemi (64-channel) 10–10 EEG system was employed for data collection. Before each experiment, electrical gel was applied on each electrode to improve the conductivity and signal to noise ratio of the data acquisition. The stimulation protocol (see Fig. 1(c)) consisted of a 2-min baseline, an 8-min tPBM, and a 3-min recovery period. In both sham and active tPBM experiments, the laser aperture was pointing at the right forehead near the electrode locations of FP2 and AF8. Both the participant and operator wore protection goggles to prevent any stray laser light from entering their eyes. While the EEG data were acquired at either 256 Hz or 512 Hz, all the 512 Hz data were down sampled to 256 Hz during data preprocessing for consistency.

Fig. 1 Experimental setup for concurrent tPBM and EEG experiment. (a) Photo of FDA-cleared, 1064-nm, continuous-wave laser (Model CG-500). (b) Schematic diagram of the experimental setup: 64 electrodes of an EEG device were attached to an international 10-10 standard EEG cap, while the subject was wearing a pair of safety goggles and retained in eye-closed resting state during the sham or active tPBM condition. The laser aperture was pointed at the right forehead of the subject with 2-cm away from the skin. (c) Shows the experimental protocol including a 2-min baseline, 8-min stimulation (with a laser power of 3.5 W for tPBM and 0 W for sham), and a 3-min recovery period.

Designed as a single-blind, cross-over study, each subject took both sham and active tPBM experiments within a period of 1 week, with a minimum of 3 days between the two experiments. The order of the sham or tPBM experiment was randomly assigned. All subjects were inquired about their experience after each experiment, including the heat sensation and potential drowsiness they perceived. Furthermore, a thermal stimulation experiment was designed to explore the impact of tPBM-associated heat sensations on human EEG signals.

For the thermo_stim experiment, a heat stimulator (Pathway model ATS, Pain and Sensory Evaluation system, Medoc Advanced Medical Systems, Israel) was employed (see Fig. 2(a)) to replicate and induce the thermal stimulation on the human forehead through an ATS mode probe. The ATS thermode can deliver temperatures ranging from 0 °C to 55 °C with a maximum rate of

8 °C/sec for the alteration of temperature. The time-dependent temperature increases on the subject's forehead induced by active tPBM were remeasured in this study using an infrared clinical thermometer (Medical Head and Ear Thermometer, Metene, England) over several human subjects; the group-averaged results were very consistent with those reported previously [14].

Fig. 2 Experimental setup and protocol for concurrent thermo_stim and EEG experiment. (a) Picture of the thermal simulator system (Pathway model with ATS thermode). (b) Schematic diagram of the experimental setup: 64 electrodes of an EEG device were attached to an international 10-10 standard EEG cap, while the subject retained eye-closed during the sham or active thermo_stim condition. The thermode was attached at the right forehead of the subject to produce heat mimicking that created in tPBM. (c) Shows the experimental protocol for thermal stimulation: including a 2-min baseline, 8-min thermo_stim, and a 2-min recovery period.

During the thermo_stim experiment, the thermode was placed at the same place same as where the tPBM was on the right forehead to simulate/mimic the thermal effect induced by tPBM, as marked in Fig. 2(b). The same 64-channel EEG device (as used in the tPBM experiment) was employed to concurrently record electrophysiological responses to the thermal stimulations. Fig. 2(c) shows the experimental protocol: It included a 2-min baseline and an 8-min thermal stimulation, followed by a 2-min recovery period. The temperature of the thermal stimulator

remained at 33 °C during the 2-min baselines for both sham and active thermo_stim. For the active stimulation, the temperature of the thermode increased from 33 °C to 41 °C following the tPBM-equivalent thermal rate ¹⁹ and was maintained at 41 °C during the remaining stimulation period. Then we removed the thermode from the forehead during the 3-min recovery period. For the sham experiment, we maintained the thermode's temperature at 33 °C throughout the 8-min period before removal of the thermode from the forehead while EEG recording lasted the entire 12-min period. In either experiment, subjects were asked to keep their eyes closed throughout the whole measurement time.

While the sampling rate of EEG during the thermo_stim experiment was 512 Hz, we also down sampled it to 256 Hz during data analysis to be consistent with that for the tPBM experiment. Further data analysis was performed only on the baseline and stimulation periods for the tPBM/sham and thermal/sham experiments, which accounts for the two minutes of baseline and the eight minutes of stimulation.

2.3. Data Analysis

Each collected EEG dataset contained 64-channels of time series corresponding to the collected large-scale neural activities at 64 scalp locations. Data from 3 participants from the tPBM group were excluded due to drowsiness and fatigue from self-reporting and experimenter's observation. Therefore, a total of 46 pairs (from 46 subjects) of EEG data under both sham and active tPBM and a total of 14 pairs (from 14 subjects) of EEG under both sham and thermo_stim were included for further data processing.

2.3.1. Data Preprocessing for EEG Time Series

The data were preprocessed using MATLAB and a MATLAB-based, open-source software package, EEGLAB. First, the acquired 64-channel EEG raw data were bandpass filtered at 0.5 -

70 Hz. A notch filter was applied to eliminate line noise at 60 Hz. Re-referencing was performed by subtracting the average of voltage signals across all the 64 electrodes from each of the EEG time series. Second, robust principal component analysis and independent component analysis were performed to remove artifacts, such as eye movements, saccades, and jaw clenches. Next, 64-electrode artifact-free time series were divided into three temporal segments: baseline, first 4 minutes (1-4 min) and last 4 minutes (5-8 min) during sham or tPBM stimulation. Specifically, (1) the last 60 seconds of the 2-min baseline before the onset of tPBM/sham were used to quantify the baseline signal as marked by $T_{\text{base-tPBM_stim}}$ and $T_{\text{base-tPBM_sham}}$, respectively; (2) the first 4 minutes (1-4 min) of the tPBM/sham stimulation were labeled by $T_{1-4\text{-tPBM_stim}}$ and $T_{1-4\text{-tPBM_sham}}$; and (3) the second 4 minutes of the tPBM/sham stimulation were represented by $T_{4-8\text{-tPBM_stim}}$ and $T_{4-8\text{-tPBM_sham}}$. The preprocessed data were then used to perform further analysis for each of the temporal segments. Likewise, the EEG data under the thermo_stim condition were preprocessed following the same steps as given above and then grouped and labeled into similar three temporal segments: $T_{\text{base-thermo_stim}}$, $T_{\text{base-thermo_sham}}$, $T_{1-4\text{-thermo_stim}}$, $T_{1-4\text{-thermo_sham}}$, $T_{4-8\text{-thermo_stim}}$, and $T_{4-8\text{-thermo_sham}}$, for both sham and active thermal stimulations.

2.3.2. EEG Power and Topography at Five Frequency Bands in Three Time Segments

For each time segment, every time series of 64 electrodes was bandpass filtered into five conventional frequency bands: delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (30-70 Hz). The respective mean EEG powers at five frequency bands were achieved with root-mean-square calculations over the chosen time segment using the MATLAB function ‘rms’ on each electrode of EEG. First, 2-sample t-tests were performed to verify that there was no significant difference in EEG powers between the baselines of tPBM and its sham experiment (i.e., $P_{\text{base-tPBM_stim}}$ and $P_{\text{base-tPBM_sham}}$). In this way, we determined mean EEG power values for each

time segment as $P_{1-4-tPBM_stim}$, $P_{1-4-tPBM_sham}$, $P_{4-8-tPBM_stim}$, and $P_{4-8-tPBM_sham}$, respectively, for each frequency band. To obtain changes from baseline, the EEG power at each frequency band during the sham and active tPBM were normalized with respect to (i.e., being divided by) their own 1-min baseline EEG powers during $T_{1-4-tPBM_stim}$ and $T_{5-8-tPBM_stim}$ for both sham and active tPBM conditions, as expressed by four terms in eq. (1) below:

$$\left(\frac{P_{1-4-tPBM_sham}}{P_{base-tPBM_sham}}\right), \left(\frac{P_{5-8-tPBM_sham}}{P_{base-tPBM_sham}}\right), \left(\frac{P_{1-4-tPBM_stim}}{P_{base-tPBM_stim}}\right), \text{ and } \left(\frac{P_{5-8-tPBM_stim}}{P_{base-tPBM_stim}}\right). \quad (1)$$

This normalization process was repeated for all 64 electrodes, enabling us to form mean power topographies during two periods of sham and active tPBM for each human subject. Consequently, percentage topographies of sham-controlled, tPBM-induced changes in mean EEG power at each frequency band (ΔP_{i-tPBM}^f in %) were obtained by subtracting the sham mean power values from those under active tPBM at each electrode for both 1-4 min and 5-8 time periods, given as below:

$$\Delta P_{i-tPBM}^f = \left(\frac{P_{i-tPBM_stim}^f}{P_{base-tPBM_stim}^f} - \frac{P_{i-tPBM_sham}^f}{P_{base-tPBM_sham}^f} \right) \times 100\%, \quad (2)$$

where i represents either 1-4 min or 5-8 min, and f indicates different frequency bands.

Similarly, the same procedures of baseline normalization/ratios and sham-subtraction were applied to the 64-channel EEG time series recorded in the thermo_stim experiments, producing percentage topographies of sham-controlled changes in mean EEG power in response to thermal stimulation at five frequency bands during the 1-4 min and 5-8 min stimulation periods for each subject, as expressed by eq. (3):

$$\Delta P_{i-thermo_stim}^f = \left(\frac{P_{i-thermo_stim}^f}{P_{base-thermo_stim}^f} - \frac{P_{i-thermo_sham}^f}{P_{base-thermo_sham}^f} \right) \times 100\%, \quad (3)$$

where i represents either 1-4 min or 5-8 min, and f marks different frequency bands.

2.3.3. Quantification of Net EEG Power Enhancements Induced by tPBM

In theory, the measured percentage changes of sham-controlled mean EEG powers at respective frequency bands, ΔP_{i-tPBM}^f , should result from both the net tPBM effect, $\Delta P_{i-tPBM}^f(net)$, and thermal effect, $\Delta P_{i-thermo_stim}^f$, namely,

$$\Delta P_{i-tPBM}^f = \Delta P_{i-tPBM}^f(net) + \Delta P_{i-thermo_stim}^f, \quad (4)$$

where i represents either 1-4 min or 5-8 min, and f marks different frequency bands. Accordingly, we would be able to determine net tPBM-induced EEG power changes (in %) at respective frequency bands, based on sham-controlled tPBM and thermo_stim EEG measurements (i.e., ΔP_{i-tPBM}^f and $\Delta P_{i-thermo_stim}^f$).

2.3.4. Statistical Analysis

Statistical analysis was performed to determine statistical significances of (1) sham-controlled tPBM effects, (2) sham-controlled thermo_stim effects, and (3) the difference between sham-controlled tPBM and sham-controlled thermal effects. For the first two statistical testing items, paired t-tests were performed between percentage topographies of mean EEG power changes (i.e., mPower in %) under the sham and active tPBM as well as under the sham and thermo_stim, respectively. Our cross-over designs for both tPBM and thermo_stim experiments justified the use of paired t-tests. For the 3rd statistical testing, two-sample t-tests were performed to identify significant difference between the mPower values of sham-subtracted tPBM and sham-subtracted thermal effects at each electrode site. Two-sample t-tests were chosen because of two different groups of participants. Furthermore, the false discovery rate (FDR) corrections were performed for comparison of topography to minimize type I errors in repeated t-tests among the 64 EEG electrodes. All statistical tests were performed at $\alpha=0.05$ after FDR correction.

Moreover, due to the unbalanced sample sizes between the tPBM and thermo_stim experiments, the effect size (d) at each electrode was calculated for the comparison between sham-subtracted tPBM and sham-subtracted thermal stimulation. The “ d ” is defined as the difference between two means divided by the standard or pooled standard deviations of the two groups. In general, $d = 0.2, 0.5, 0.8$, and 1.2 are considered a small, medium, large, and very large effect size, respectively.

3. Results

We reported results based on several statistical comparisons. First, we looked at the sham-controlled tPBM-induced effects on EEG powers at delta, theta, alpha, beta, and gamma bands. Second, we investigated the sham-controlled thermo_stim effects on EEG powers at the same five frequency bands. Last, we evaluated the differences between the sham-controlled tPBM versus thermal effects for the respective frequency bands.

3.1. Sham-controlled tPBM-induced Alterations in Mean EEG Power and Topography

The top row of Fig. 3 illustrates the baseline-normalized, sham-subtracted topographies for EEG percentage changes in mPower (i.e., %) by tPBM at all five frequency bands averaged over $n=46$ participants. The second row of Fig. 3 shows statistical t-maps (obtained with paired t-tests and FDR correction) during the first four minutes (T1-T4) and the second four minutes (i.e., T5-T8) of the tPBM/sham stimulation for the respective frequencies.

The results clearly showed significantly decreased mPower at anterior and posterior cerebral regions during the first 4-min of tPBM in the delta band. However, this decreased delta power was not persistent after the first four minutes of tPBM. More important, significantly increased alpha and beta mPower distributions were shown on multiple sites of the human scalp, particularly during the second 4-min of tPBM. As seen in this figure, during the last half tPBM period, both

the sham-controlled increase in mPower topography and t-map revealed anterior-posterior power-enhancement patterns for the alpha band. A smaller number of significant increases in beta mPower occurred, located at central and posterior regions of the scalp. No significant difference in mPower was observed between tPBM and sham in theta and gamma bands.

Fig. 3 Group-level (n=46), baseline-normalized, sham-controlled topographies for EEG mean power (mPower) alterations induced by tPBM. The first row shows the mean power changes during the first 4-min (T1-T4) and last 4-min (T5-T8) tPBM. The second row presents the corresponding statistical T-topographies for the comparison between tPBM versus sham stimulation, with paired t-tests at $\alpha = 0.05$ after FDR correction for T1-T4 and T5-T8. Solid blue and red dots mark the sites that had significant changes in mPower with respect to the sham stimulations.

3.2. Sham-controlled Thermo_stim-induced Changes in Mean EEG Power and Topography

Following the same data presentation style as that in Fig. 3, The top row of Fig. 4 illustrates the baseline-normalized, sham-subtracted topographies for EEG mPower alterations (in %) by thermo_stim at all five frequency bands averaged over n=14 participants. The second row of Fig. 4 shows statistical t-maps (obtained with paired t-tests and FDR correction) during the first and second halves of the stimulation period. This figure clearly demonstrates that mean EEG powers in alpha and beta frequencies were significantly reduced, particularly during the last half period of thermal stimulation relative to the sham condition. Specifically, an anterior-posterior reduction

292 took place in the alpha mPower while global decreases occurred in the beta mPower. However,
 293 the delta, theta, and gamma bands did not show any significant changes in mPower.

294 **Fig. 4** Group-level (n=14), baseline-normalized, sham-controlled topographies for EEG mean
 295 power (mPower) alterations induced by thermo_stim. The first row shows the mean power changes
 296 during the first 4-min (T1-T4) and last 4-min (T5-T8) tPBM. The second row presents the
 297 corresponding statistical T-topographies for the comparison between thermo_stim versus sham
 298 stimulation, with paired t-tests at $\alpha = 0.05$ after FDR correction for T1-T4 and T5-T8. Solid blue
 299 dots mark the sites that have significant decreases in mPower with respect to the sham stimulations.

300 3.3. Net EEG Power Enhancements Induced by tPBM Excluding Thermal Effects

301 As expressed by eq. (4), we would be able to quantify net tPBM-induced EEG mPower increases
 302 at the group level by subtracting the sham-controlled thermo_stim mPower topography (top row
 303 of Fig. 4) from the sham-controlled tPBM mPower topography (i.e., top row of Fig. 3). This
 304 operation leads to Fig. 5(a), which illustrates the group-level difference of the baseline-normalized,
 305 sham-subtracted spatial distributions of mPower between tPBM and thermal stimulations, i.e.,
 306 $\Delta P_{i-tPBM}^f(net)$, during $i=T1-T4$ and $T5-T8$ at $f=$ delta, theta, alpha, beta, and gamma bands. Figs.
 307 5(b) and 5(c) are two statistical topographies for t-test (T-map) and effect sizes (ES) between sham-
 308 controlled tPBM effects and sham-controlled thermal effects. The t-tests were based on 2-sample
 309 t-tests at $\alpha = 0.05$ with FDR correction.

As shown in Figs. 3 and 4, thermal stimulation created the opposite effects on EEG mPower patterns in alpha and beta bands with respect to those by tPBM. This opposite effect gave rise to a larger difference between the two types of stimulations (i.e., $\Delta P_{i-tPBM}^f(net)$), as illustrated by Fig. 5(a), mainly at $f = \alpha$ and β bands. Statistically, the two-sample t-tests confirmed that the alpha and beta mPower changes induced by tPBM were significantly higher with larger effect sizes than those by thermo_stim at anterior-posterior regions during the entire 8-min stimulation period. However, no significant photobiomodulation effects took place in delta, theta, and gamma bands.

Fig. 5 (a) Group-level difference in mPower maps between baseline-normalized, sham-subtracted topographies from tPBM (n=46) and thermo_stim (n=14) experiments in all five frequency bands for the first 4-min (T1-T4) and second 4-min (T5-T8) of the stimulation period. (b) it shows statistical t-map topographies obtained from two-sample t-tests with red dots marking electrode sites having significant increases in ePower ($\alpha = 0.05$ with FDR correction) by tPBM with respect to thermo_stim. (c) Shows effect sizes (ES) for comparing tPBM and thermal effects.

4. Discussion

We recorded scalp EEG *in vivo* before, during, and after tPBM/sham from 49 human subjects under the eyes-closed resting state. Furthermore, we generated the same temperature enhancement induced by tPBM on human foreheads using a thermal generator. For the latter, we recorded EEG *in vivo* from 14 human subjects before, during, and after the thermal stimulation with its

corresponding sham experiment. Baseline-normalized EEG power alterations were compared between tPBM laser-illumination and its sham experiments. More importantly, the sham-subtracted, tPBM-induced EEG mPower topographies from 46 participants were compared at the group level with the sham-subtracted thermal-induced EEG mPower taken from another group of 14 human subjects. In this way, we rigorously investigated (1) how tPBM modulates brain rhythm powers under the eyes-closed resting state, (2) whether and how thermal stimulation modulates brain rhythm powers under the eyes-closed resting state, and (3) whether thermal stimulation effect could be removed to recover/obtain net tPBM effects on the brain rhythm powers.

4.1. tPBM-induced Alterations in EEG mPower at Alpha, Beta, and Delta Bands

Fig. 3 illustrates the sham-controlled topographic mPower increments induced by tPBM laser-illumination under eyes-closed resting state. During the last half period of tPBM, significantly enhanced power values were clearly observed in the anterior-posterior regions for alpha oscillations and in the central and posterior regions for beta oscillations. Overall, these results are in good agreements with those taken under eyes-open resting state, as we reported before [15, 16].

While the alpha power is believed to be related to wakefulness²⁸, it is also commonly associated with cognition-related brain functions such as memory encoding, attention, and brain network synchronization and interaction²⁹⁻³¹. Moreover, studies indicate that cortical alpha waves are engendered due to the collaboration of thalamocortical and cortico-cortical interactions³². With several hundred human subjects, previous studies have demonstrated that 1064-nm laser enabled significant behavioral improvements in cognitive functions using the same experimental protocol^{3,9-11,33}. Putting all these results together, we speculate that improvement of human cognition by tPBM may be closely associated with alpha power increases and potential stimulation to the

anterior-posterior network, which is an executive network that assists rapid instantiation of new tasks by interacting with other control and processing networks ³⁴.

In addition, the literature has shown that beta activation is responsible for maintaining concentration or focus. Several studies have presented evidence that improved beta waves are a sign of better cognitive capacity ^{35,36}. Hostovecky et al. demonstrated that beta waves are essential to neurofeedback activities ³⁵. Another study demonstrated that beta activation is an indication of cognitive processes with higher concentration by conducting rejection tasks or mental arithmetic tasks, both of which require attention and internal processing ³⁶. On the other hand, since beta waves are a sign of somatosensory processing, beta activation might be responsible for the heat sensation from the tPBM laser ³⁷. In our case, however, the beta response to heat would most probably be limited to the left central cortex near the somatosensory region because the right forehead was illuminated by the tPBM laser ³⁸. Thus, thermal sensation would not account for the observation of beta activation beyond the somatosensory area (Fig. 3). There must exist another mechanism of action for beta mPower increase, as discussed in Section 4.5.

Besides increasing alpha and beta mPower, tPBM reduced delta power during the first 4 minutes of stimulation. While the power of delta waves has been widely related to human sleep, the power of delta oscillation at resting state has also been linked to cognitive functions. For example, Bablioni et al. reported that better cognitive performance is associated with a decreased power of delta waves ³⁹. During initial 4-min tPBM, the significant decrease in delta power at resting state might potentially enhance cognitive functions. Furthermore, according to the literature, increased delta wave has been associated with fatigue ^{40,41}, depression ⁴², and addiction ^{43,44}. The modulatory effects of tPBM on delta waves are opposite to the abnormalities found in patients with emotional and cognitive disorders. Thus, tPBM might have a promising prospect to

reverse the changes seen in various psychiatric and psychological conditions⁴⁰⁻⁴⁴. However, this expectation needs to be further investigated with an optimal selection of stimulation length and intervention designs because of time-dependent patterns in delta mPower changes.

4.2. Alterations in EEG mPower by Thermo_stim Are Opposite to Alterations by tPBM

As shown in Fig. 4, the thermal stimulation following the equivalent temperature rise given by tPBM, induced significant decreases of global alpha and beta powers, meaning strong desynchronizations of alpha and beta waves across the entire scalp. These observations are very consistent with previous EEG studies using non-noxious thermal stimuli⁴⁵ and noxious thermal stimuli^{46,47}. However, few EEG studies have reported effects of nonpainful thermal stimuli given on the human head since most of thermal stimulation sites were on peripheral locations^{45,47-50}. One study on tonic pain using continuous EEG to predict subjective pain perception observed significant decreases in alpha (7-10 Hz) power during the stimulation and suggested that this decrease was due to an augmented activity of cortico-cortical and thalamocortical feedback loops⁵⁰. There are numerous EEG-based publications to investigate mechanisms of pain, but they are beyond the focus of this study. The major observation and conclusion drawn from Figs. 3 and 4 were that the trends and topographic patterns of percentage changes in EEG mPower induced by thermo_stim were opposite to those by tPBM. In other words, these results confirmed unambiguously that the percentage changes in EEG mPower at alpha and beta bands by sham-controlled 1064-nm tPBM could not stem from the thermal impact of the laser used in tPBM.

4.3. Net tPBM Effects Excluding Thermal Effects

Because of the opposite trends in percentage changes of EEG mPower between the two types of stimulations, as large as ~25% increases in alpha mPower (Fig. 5(a)) and large effect sizes of 0.8 or larger (Fig. 5(c)) were achieved in an anterior-posterior pattern under tPBM during the entire 8-

min period relative to the sham. Furthermore, the two-sample t-tests (Fig. 5(b)) statistically proved that the two stimulation methods by laser and heat created distinct changes of electrophysiology in alpha and beta frequencies across most of the electrodes sites. This conclusion is in excellent agreement with one of our previous studies, which presented that the thermal effect was independent and opposite to the tPBM impact on cerebral hemodynamic oxygenation ([HbO]) and metabolic oxidation ([CCO]) near the tPBM site¹⁹. Taking all these observations together emphasized that the detected alterations in alpha and beta EEG mPower globally, [HbO] locally, and [CCO] locally resulted from only 1064-nm tPBM, not from thermal sensation. The enhancement of anterior-posterior synchronization by tPBM at alpha and beta waves might be due to another mechanism or electrophysiological path, which will be explored and examined in our future studies.

In addition, the laser/thermal stimulation applied in this study has been proved safe, non-painful, and often little perceptible to human subjects at the laser power of $\sim 250 \text{ mW/cm}^2$. A study conducted on a rabbit brain using CW and pulsed lasers demonstrated that the heat generated by a laser with less than 750 mW/cm^2 does not cause tissue damage⁵¹.

4.4. tPBM-induced EEG mPower Changes in Eyes-open and Eyes-closed Resting State

One of the objectives of this study was to compare the tPBM-induced effects between eyes-open and eyes-closed resting state because EEG signals have been shown to behave differently between these states^{26,52}. In the eyes-open resting state, the human brain encounters many visual stimuli and activates the visual information processing networks/paths. However, those processes and pathways are suppressed during the eyes-closed resting state due to the blockage of visual information input⁵², indicating the distinct emphasis of brain networks and processes in these two

states. This is why the eyes-closed state naturally creates higher absolute powers of alpha wave compared to the eyes-open resting state on healthy humans ⁵².

However, the abovementioned difference between the eyes-open and eyes-closed state would not necessarily affect the comparisons of the tPBM-induced EEG mPowers at alpha and other frequency bands between the two states in the current study because the comparisons were taken after baseline normalization and sham subtraction for each participant. In this way, the sham-controlled, tPBM-evoked mPowers under the eyes-closed state given in Fig. 3 can be fairly compared with those under the eyes-open state as reported in Ref. ²⁰. Indeed, they both show very agreeable trends and patterns of alterations of mPowers at both alpha and beta bands. Moreover, the increases in alpha/beta mPower are also compatible with several other published tPBM studies. For example, using an 810-nm pulsed LED system, Zomorodi et al. reported reduction in delta power and improvement in alpha and beta power after 20-min tPBM ²⁴. These results emphasized the robustness of alpha and beta waves neuromodulation by tPBM.

Nevertheless, there were a couple of slight differences in the global distribution of power changes between the two resting states. For example, tPBM resulted in more beta power activation in the central regions with the eyes-closed state (Fig. 3) and in the occipital areas with eyes-open state [15]. Moreover, significant deactivation in the delta power was not observed in the eyes-open experiment [15]. Such differences might have resulted from subtle differences between the experimental protocols. In our previous eyes-open study, the stimulation was 11 min with 2.2-W optical power for tPBM delivered by the same laser. In the current study, to minimize the possibility of subjects being drowsy during the experiment, we designed an 8-min stimulation at 3.5 W, with the same total optical energy as in the 11-min tPBM study. The current study utilized a little higher laser power compared to the previous one, possibly leading to more heat/warm

sensation by the participants. As shown in Fig. 4, the cross-subject topographies of thermal stimulation may reveal potential thermal sensation because of the reduced delta power at the frontal region.

4.5. Methods of EEG Power Analysis, Post-Stimulation, and Frontoparietal Network

We employed the root-mean-square (RMS) method to quantify EEG powers during three separate temporal segments (i.e., 2-min baseline, 1-4 min, and 5-8 min during tPBM) at each of the five frequency bands. Conventionally, EEG powers are quantified by converting the time-domain data to the frequency-domain power spectrum density (PSD) via Fourier transform, followed by spectral average within the selected spectral range. Indeed, we have tested the results using both RMS and PSD for several EEG time series under sham and active tPBM; the results confirmed that the outcomes from both methods were in excellent agreement, as shown in supplementary material, Fig. S1.

In this study, we did not present post-stimulation results in either tPBM or thermo_stim experiments. Our focus was to address (1) whether there existed any thermal impact, created by 1064-nm tPBM equivalent, on the changes of EEG powers at five frequency bands, and (2) whether the tPBM-evoked net increases in EEG powers at alpha and beta waves were unaffected after removal of the thermal impact on EEG powers. Thus, it was not necessary to inspect results in the post-stimulation period.

Overall, our results presented strong and significant enhancement by tPBM in EEG power or synchronization for frontal-parietal alpha and beta oscillations. It is acknowledged³⁴ that the frontoparietal network is a flexible hub for cognitive control and “a distinct control network, in part functioning to flexibly interact with and alter other functional brain networks. This network coordination likely occurs in a 4 Hz to 73 Hz θ/α rhythm, both during resting state and task state.”

Thus, it is reasonable to speculate that the ability of tPBM to strongly modulate or synchronize alpha and beta oscillations in the frontoparietal network may be closely associated with or serves as the electrophysiological mechanism of action that tPBM is able to significantly improve human cognition observed by our group [3, 7-9] and others^{4,7,8,24}.

Moreover, according to³⁴, “precision mapping of individual human brains has revealed that the functional topography of the frontoparietal network is variable between individuals, underscoring the notion that group-average studies of the frontoparietal network may be obscuring important typical and atypical features.” This notion explains why the observed spatial distribution of enhanced EEG alpha and beta mPower was rather spread across frontal-parietal regions, in addition to a systematic backwards shift of the EEG cap.

4.6. Limitations and Future Work

This study also had several drawbacks and thus have opportunities for future work. First, the thermal stimulation was given based on contact delivery from the thermode to the human forehead, whereas equivalent heat emitted from the laser in tPBM was non-contact. Also, the total area of thermal stimulation by the thermode was relatively smaller than the site of tPBM laser aperture. Second, the two sample sizes for tPBM and thermo_stim experiments were too unbalanced with the thermal group having too fewer participants (n=14), which may cause inaccurate or insufficient statistical conclusions. Last, the international 10-10 EEG cap system in this study was not strictly followed since a clear area with 4-cm in diameter was needed for tPBM light delivery on the right forehead. Thus, the 64-channel EEG cap was shifted about 1-2 cm backwards, which would create ~1-2 cm location errors in standard 64-channel topographies given in Figs. 3-5. As for future work, a non-contact heat generator may be a better option to replicate the findings in this study with a larger number of human subjects. To obtain/mark more accurate mPower topography with correct

electrode locations, a 3-dimensional digitizer can be utilized to quantify exact locations or coordinates of the 64 electrodes with respect to those in the 10-10 system on each subject's head, followed by modification or correction of the EEG power topography using interpolation and extrapolation based on the standard 10-10 electrode system. Last, more quantitative analysis on network connectivity and directional information flow will be taken to substantiate our expectation that tPBM indeed modulates the frontoparietal network significantly during and post tPBM.

5. Conclusion

This study demonstrated that baseline-normalized, sham-controlled tPBM with a 1064-nm laser given on the right forehead of healthy human subjects neuromodulated delta, alpha, and beta oscillations in eyes-closed resting state. Moreover, we demonstrated that thermal stimulations would generate opposite percentage changes in alpha and beta oscillation powers with respect to those by tPBM. After careful two-sample t-tests, we proved our hypothesis that tPBM-induced increases in anterior-posterior EEG powers at alpha and beta bands remained statistically significant during the eyes-closed resting state, similar to those during the eyes-open resting state, after removal of tPBM-associated thermal effects. The observed strong enhancement by tPBM on alpha and beta oscillations in the anterior-posterior regions may be the underlying electrophysiological mechanism of action to explain why tPBM enables to improve human cognition.

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Author contributions

Drs. Wanniarachchi, Wang, and Liu designed the experimental protocol. Drs. Wanniarachchi, Wang, and Wu conducted the study, including human subject recruitment, instrument setup and calibration, data collection, and data analysis. Drs. Wanniarachchi and Wang prepared the manuscript draft with important intellectual input and supervision from Dr. Liu. Drs. Liu and Gonzalez-Lima edited and finalized the manuscript. All authors approved the final manuscript. The National Institute of Mental Health provided funding for the study to Drs. Liu and Gonzalez-Lima.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

Experimental setup for concurrent tPBM and EEG experiment. (a) Photo of FDA-cleared, 1064-nm, continuous-wave laser (Model CG-500). (b) Schematic diagram of the experimental setup: 64 electrodes of an EEG device were attached to an international 10-10 standard EEG cap, while the subject was wearing a pair of safety goggles and retained in eye-closed resting state during the sham or active tPBM condition. The laser aperture was pointed at the right forehead of the subject with 2-cm away from the skin. (c) Shows the experimental protocol including a 2-min baseline, 8-min stimulation (with a laser power of 3.5 W for tPBM and 0 W for sham), and a 3-min recovery period.

Figure 2

Experimental setup and protocol for concurrent thermo_stim and EEG experiment. (a) Picture of the thermal simulator system (Pathway model with ATS thermode). (b) Schematic diagram of the experimental setup: 64 electrodes of an EEG device were attached to an international 10-10 standard EEG cap, while the subject retained eye-closed during the sham or active thermo_stim condition. The thermode was attached at the right forehead of the subject to produce heat mimicking that created in tPBM. (c) Shows the experimental protocol for thermal stimulation: including a 2-min baseline, 8-min thermo_stim, and a 2-min recovery period.

Figure 3

Group-level (n=46), baseline-normalized, sham-controlled topographies for EEG mean power (mPower) alterations induced by tPBM. The first row shows the mean power changes during the first 4-min (T1-T4) and last 4-min (T5-T8) tPBM. The second row presents the corresponding statistical T-topographies for the comparison between tPBM versus sham stimulation, with paired t-tests at $\alpha = 0.05$ after FDR correction for T1-T4 and T5-T8. Solid blue and red dots mark the sites that had significant changes in mPower with respect to the sham stimulations.

Figure 4

Group-level (n=14), baseline-normalized, sham-controlled topographies for EEG mean power (mPower) alterations induced by thermo_stim. The first row shows the mean power changes during the first 4-min (T1-T4) and last 4-min (T5-T8) tPBM. The second row presents the corresponding statistical T-topographies for the comparison between thermo_stim versus sham stimulation, with paired t-tests at $\alpha = 0.05$ after FDR correction for T1-T4 and T5-T8. Solid blue dots mark the sites that have significant decreases in mPower with respect to the sham stimulations.

Figure 5

(a) Group-level difference in mPower maps between baseline-normalized, sham-subtracted topographies from tPBM (n=46) and thermo_stim (n=14) experiments in all five frequency bands for the first 4-min (T1-T4) and second 4-min (T5-T8) of the stimulation period. (b) it shows statistical t-map topographies obtained from two-sample t-tests with red dots marking electrode sites having significant increases in ePower ($\alpha = 0.05$ with FDR correction) by tPBM with respect to thermo_stim. (c) Shows effect sizes (ES) for comparing tPBM and thermal effects.

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