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Effects of stimulating frequency of NIR LEDs light irradiation on forehead as quantified by EEG measurements

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Near-infrared (NIR) light has been shown to produce a range of physiological effects in humans, however, there is still no agreement on whether and how a single parameter, like the flicker frequency of NIR light, affects the brain. An 810 nm NIR LED was used as the stimulator. Fifty subjects participated in this experiment. Forty subjects were randomly divided into four groups. Each group underwent a 30-minute NIR LED radiation with four different frequencies (i.e., 0 Hz, 5 Hz, 10 Hz and 20 Hz, respectively) on the forehead. The remaining 10 subjects formed the control group, in which they underwent a 30-minute rest period without light radiation. EEG signals of all subjects during each test were recorded. Gravity frequency (GF), relative energy change, and sample entropy were analyzed. The experimental groups had larger GF values compared to the control group. Higher stimulation frequency would cause larger growth of GF ($F = 14.75$, $P < 0.001$). The amplitude of alpha waves relative energy increased, while theta waves decreased remarkably in the experimental groups ($p < 0.02$), and the extent of increase/decrease was larger at higher stimulation frequency, compared to that of the control. Sample entropy of electrodes in the frontal areas were much larger than those in other brain areas in the experimental groups ($p < 0.001$). Larger frequency of the NIR LED light would cause more distinct brain activities in the stimulated areas. It indicates that NIR LED light may have a positive effect on modulating brain activity. These results may help improve the design of photobiomodulation treatments in the future.

Keywords: Photobiomodulation; LED light therapy; near-infrared light; gravity frequency; relative energy.

1. Introduction

Photobiomodulation (PBM) is a treatment method in which irradiation with certain wavelengths of red or near-infrared (NIR) light has been shown to produce a range of physiological effects in cells, tissues, animals and humans.[1] Low-intensity

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phototherapy modulates numerous cellular functions. Low-power lasers and light-emitting diodes (LEDs) are widely used therapeutic tools for ischemic wounds, infection, and other soft tissue injuries.[2] In recent years, LEDs have become commonly used. Advantages of LEDs include no laser safety considerations, ease of home use, ability to irradiate a large area of tissue at once, possibility of wearable devices, and much lower cost per mW.[1] Compared to laser, NIR LED light has gained more attention for its ability to activate anti-inflammatory processes and is now widely used in veterinary medicine to treat sprains, bone fractures, and to speed the healing of wounds.[3] Over the last 10 years, there has been a lot of studies focusing on whether the transcranial NIR LED light has a regulatory effect on electrical activity in the brain, and results from these studies have shown the neuromodulatory effect of transcranial NIR.[4] Nevertheless, the light-source type is not the only parameter that must be considered when using PBM. Parameters like wavelength, energy density, power density and the pulse structure may influence the PBM outcomes as well. For now, there is no consistent results on effects of these factors on brain activities in clinical applications.[5] In this study, we are going to explore whether and how one of these factors, like the flicker frequency of NIR light, affects the brain.

Research shows that the mechanism of NIR LED phototherapy depends on the absorption of photons within a specific wavelengths by cytochrome c oxidase (CCO).[6] CCO is the most important chromophore that absorbs light. Delpy and Cope[7] showed that over 50% of the light absorption between 800 nm and 850 nm was due to CCO, it has two absorption bands, one in the red spectral region ($\sim$ 660 nm) and another in the NIR spectrum ($\sim$ 800 nm), which are the wavelengths most commonly used in PBM. In addition to increasing adenosine triphosphate (ATP) production, NIR LED light can modulate early-response genes, activate reactive oxygen species, induce synaptogenesis, and stimulate cell proliferation. NIR wavelengths, such as 810 nm, stimulate mitochondrial activity and ATP production.[8,9]

In some clinical trials, 808 nm NIR light was used to illuminate the human scalp continuously for 40 min, which has been shown to be effective in treating traumatic brain injury.[10] A later case series of 11 patients treated with a similar LED device which delivered NIR with a wavelength of 870 nm at a fluence of 13 J/cm,[2] for 20 min each session, demonstrated improved attention, inhibition, and verbal memory based on psychological testing after 18 sessions.[11] Using an 810 nm NIR light pulse at 40 Hz, Zomorrodi *et al.* found that a single session of tPBM significantly increased the power of the subjects' alpha, beta and gamma frequencies and decreased the power of delta and theta frequencies in the resting state.[12] Blanco *et al.*[13] used a specific wavelength laser (1064 nm) to explore whether transcranial laser stimulation improved the performance of the Wisconsin Card Sorting Task (WCST). These results showed that subjects receiving laser treatment had fewer errors than the placebo controls and showed improved set-shifting ability. Disner *et al.*[14] studied the efficacy of PBM delivery to the head (above the right prefrontal cortex) combined with attention bias modification (ABM) in the treatment of 51 patients with depression. Patients were treated with wavelengths of 1064 nm, irradiance of 250 mW/cm^2 for 4 min. They found that PBMT led to greater symptom improvement, which indicated that ABM could be improved by adjunctive interventions. Wu *et al.*[15] induced traumatic brain injury (TBI) in mice and treated the animals using 660, 730, 810, or 980 nm, single dose treatment of 36 J/cm^2 using an irradiance of 15 mW/cm^2, 4-minute duration, 4 h after injury. They found that mice with moderate to severe damage showed significant improvements only when using 810 nm.

Important researches have been made about the amount of energy that can reach the target tissue and the radiation time. For example, 0.03–0.3 J/cm^2 of energy delivered to a monolayer of cells in tissue culture was enough to activate transcription factors, while two orders of magnitude greater energy was required to invoke change in the rodent joint with inflammation.[16] Animal studies of low-level infrared light therapy have shown demonstrable neurodegeneration and repair.[17] Salehpour *et al.*[18] studied brain mitochondrial function after D-galactose-induced mitochondrial dysfunction in mice. Animals were treated with wavelengths of 810 nm at two different fluences: 4 and 8 J/cm^2; 10 Hz and 4.75 W/cm^2. They found poor results at 4 J/cm^2 and an amelioration of the aging induced mitochondrial dysfunction with 8 J/cm^2. These studies have shown the effects of the amount of energy and radiation time on the brain activities.

Researchers also stated that the variation of the stimulation frequency has a direct effect on brain activity. Hamblin *et al.*[19] concluded that pulsed light maybe superior to continuous wave (CW) light, particularly for post stroke management. However, the clinical application parameters and protocols of PMB have not yet reached agreement and what the effects of different frequencies of the NIR light may have on the brain activities is largely unknown.

Brain activities can be quantified by electroencephalography (EEG) measurements, which has been shown to be a viable tool based on several measurable parameters, such as gravity frequency, the sample entropy, relative of energy, etc.[20] Gravity frequency of EEG is an important factor, which has a strong correlation with the excitability of the brain.[21] With the progression of brain fatigue, the center of gravity frequency of EEG decreases, which indicates the decline of subjects' alertness level. In addition, the sample entropy is a measure of complexity reflecting the degree of disorder of the time series signals and the degree of chaos of the multi-frequency components. The larger the sample entropy, the more complex the signal would be.

Thus, the goal of this study is to determine whether and how different frequencies of the NIR LED light affect brain activity by evaluating the relative energy and gravity frequency of EEG signals. This study may shed new light on the mechanisms of NIR LED light stimulation with varying frequency on brain injury treatment.

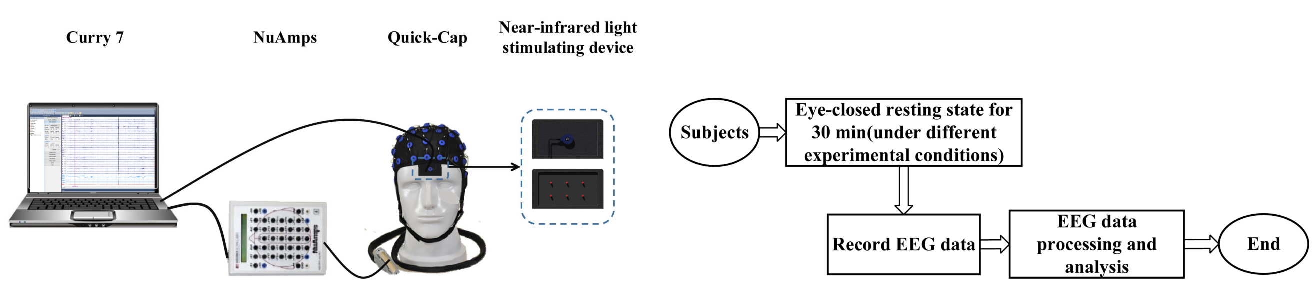
2. Material and Methods

2.1. *Subjects*

Fifty right-handed person (aged between 22 and 26 years old), all healthy, both physically and mentally, participated in this experiment. They were randomly recruited from Nanjing University of Aeronautics and Astronautics (NUAA), China. All experiments were carried out in accordance with institutional guidelines of NUAA. The subjects were informed about the process and precautions of the experiment before the tests, and written consent from all subjects were given. All experimental protocols were approved by the Ethics Committee of NUAA.

2.2. *Experimental protocol*

Figure 1(a) shows the schematic diagram of the experimental system, which includes a stimulation part and a recording part. The simulation part consists of a small area of LED array and a temperature sensor. The LED array has two rows of LED with a peak wavelength of 810 nm and a full bandwidth of 40 nm composition. When the LED array's output power density is 30.65 mW/cm^2, the influence rate is 2.19 J/(cm^2· min). The duty cycle of PBM device designed in this paper is 50%. LED is fixed by a black opaque soft plastic material and the size of the stimulating module is 3.5 cm×7 cm (Fig. 1). The frequency of the NIR LED light is tuned with a computer program in the range of 0–30 Hz. During the experiment, the stimulating module is fixed by a medical tape onto the forehead of the subjects. The brain may suffer from many different disorders, such as traumatic events, degenerative diseases and psychiatric disorders, and so on. There is some evidence that all these seemingly diverse conditions can be beneficially affected by applying light to the head.[4,10–12] There is even the possibility that PBM could be used for cognitive enhancement in normal healthy people.[27,34] In this transcranial PBM (tPBM) application, near-infrared (NIR) light is often applied



(a) (b)

Fig. 1. (a) The schematic diagram of the experimental system. (b) The flow chart of the study.

to the forehead because of the better penetration (no hair, longer wavelength).[10,12,27,32]

During the experiment, EEG signals were collected in real-time by the NuAmps electroencephalograph. Thirty-two channels of Ag/AgCl electrodes were placed on the scalp according to the expanded international 10–20 montage system for EEG measuring and recording. Two mastoid electrodes at both sides of the earlobes were used as the alternative references, and Fpz was set as the ground electrode. The sampling rate was set to 1 KHz, and the skin impedance of each electrode was less than 5 KΩ. The experiment was carried out in a quiet, dark room at a temperature of 24°C.[18]

Fifty subjects were randomly divided into five groups (10 people each group), including one control group and four experimental groups. The stimulating module is fixed by a medical tape onto the forehead of the subjects. The stimulating module on the forehead of the control subjects did not work. Figure 1(b) shows the flow chart of the study. The LED flicker frequencies of the four stimulation experiments were 0 Hz, 5 Hz, 10 Hz, and 20 Hz, respectively. Each experiment lasted for about 30 min and EEG signals were acquired throughout the process. During the stimulation, all subjects were in eye-closed resting state and tried to minimize unnecessary movements. Nodding and body movements were recorded by video in real time.

2.3. *Data processing*

The raw EEG data can be contaminated with a lot of high-frequency and low-frequency noise. High frequency noise is mainly caused by atmospheric thermal noise and power frequency noise, while low frequency noise is mainly caused by respiration and heartbeat. All EEG signals were corrected basing on the baseline. Since the absolute value of the EEG signal amplitude is less than 150 uV, values greater than 150 uV were treated as artifacts and were eliminated.[12,22] Based on real-time video data, the noise signal caused by unnecessary movements could be eliminated manually. After that, all signals were filtered with 0.01–30 Hz band-pass filtering. All 30 channels of EEG data were subjected to the IIR digital filter implemented in the EEGLAB software package.

The EEG data of 30 channels every one-minute duration were processed respectively for gravity frequency, and then the mean gravity frequency of 30 channels was calculated. The relative energy of each two-minute data segment of the 30 channels was calculated separately, and then the average of the relative energy of the 30 channels was calculated. The sample entropy of different channels of the data in the first one minute of the experiment and the data in the last one minute of the experiment were calculated, respectively.

2.3.1. *Gravity frequency calculation*

The EEG data of all subjects in each group were averaged and gravity frequency of EEG signal was calculated based on Eq. (1):

$$\mathrm{GF} = \frac{\sum_{\omega=\omega_1}^{\omega_2} \hat{p}(\omega)\omega}{\sum_{\omega=\omega_1}^{\omega_2} \hat{p}(\omega)}, \tag{1}$$

where $\hat{p}(\omega)$ is the EEG power spectral density,[23] ω_1, ω_2 are the lower and upper limits of the total frequency ($\omega_1 = 0.5$ Hz and $\omega_2 = 20$ Hz in this paper).

2.3.2. *Relative energy based on different wavelength*

The data (30 min long) was divided into 15 shorter segments (each with a two-minute duration) on average and each two-minute data was sectioned into one epoch. All the selected epochs were analyzed using Fourier Transform. After that, we obtained four frequency wavebands, which were named A (0.01–2 Hz), B (4–6 Hz), C (9–11 Hz), and D (19–21 Hz), belonging to delta, theta, alpha, beta waves, respectively. The energy of A, B, C, and D could be calculated from the following equation:

$$E_j = \sum_i x(i)^2, \quad j = 1, 2, 3, 4, \tag{2}$$

where $x(i)$ represent the amplitude of frequency wavebands. E_1, E_2, E_3, and E_4 are the energy of A, B, C, and D, respectively. The relative energy RE_j was calculated as follows:

$$RE_j = E_j / \sum_{k=1}^{4} E_k, \quad j = 1, 2, 3, 4. \tag{3}$$

In addition, relative energy difference values with time were calculated by the following equation:

$$y = S_i - S, \quad i = 1, 2, 3, 4, \tag{4}$$

where S represents the value of four parameters of the control group, S_i represents the value of four parameters (i.e., A, B, C, D) in four different experimental groups, $i = 1, 2, 3, 4$ represents the experimental group corresponding to flicker frequency of 0 Hz, 5 Hz, 10 Hz and 20 Hz.

2.3.3. *Sample entropy calculation*

The sample entropy of EEG signals is the probability of generating new patterns of EEG signals. The larger the value is, the more likely the new patterns will be generated, and the more complex the EEG signals are. The more the brain is engaged in the current things, the greater the representative concentration.[24] The original data is set as $u(1)$, $u(2), \ldots, u(N)$ (N points in total). The dimension vectors are composed in the sequence of serial numbers: from X_m (1) to $X_m(N-m)$, as shown in Eq. (5):

$$\mathrm{X_m}(i) = [u(i), u(i+1), \ldots, u(i+m-1)], \quad (5)$$

where $i = 1 \sim N - m$ and these values represent u values starting from i (m in total). The distance $\mathrm{d}[X_m(i), X_m(j)]$ between the vectors $X_m(i)$ and $X_m(j)$ is defined as the maximum value of the difference between the corresponding elements, shown as follows:

$$\mathrm{d}[X_m(i), X_m(j)] = \max(|u(i+k) - u(j+k)|), \quad (6)$$

where $k = 0 \sim m-1, i, j = 1 \sim N-m, j \neq i$. Set the threshold value as r, when $i \leq N - m$, count the number of $\mathrm{d}[X_m(i), X_m(j)]$ less than a (template matching number) and the ratio of 1 to $\mathrm{N} - m - 1$, which is recorded as $\mathrm{B}_\mathrm{r}^m(i)$, calculated according to Eq. (7):

$$\mathrm{B}_\mathrm{r}^m(i) = N^m(i)/(N - m - 1). \quad (7)$$

Then based on Eq. (7), the average value for all i was calculated as follows:

$$\mathrm{B}^m(r) = (N-m)^{-1} \bullet \sum_{i=1}^{N-m} B_r^m(i). \quad (8)$$

Increase the dimension to the vector of $m+1$ according to the sequential order of the number, and calculate $\mathrm{X_{m+1}}(i)$, $\mathrm{d}[X_{m+1}(i), X_{m+1}(j)]$, $\mathrm{B}_\mathrm{r}^{m+1}$ and $\mathrm{B}^{\mathrm{m+1}}(r)$ according to Eqs. (5)–(8). Based on Eq. (8), the sample entropy is calculated as follows:

$$\mathrm{S}ampEn(m, r, N) = -\ln[B^{m+1}(r)/B^m(r)]. \quad (9)$$

When calculating the *SampEn*, the three parameters m, r and N should be selected first. Based on experience and experimental results, take $m = 2$, $r = 0.2SD$ (SD is the standard deviation of the sequence). In this paper, the data of 1 min was used for calculation. Since the sampling frequency is 1000 Hz, $N = 60000$ was selected.

2.3.4. *Statistical analysis*

One-way ANOVA was performed to the gravity frequency, the relative energy and sample entropy to identify significant differences among A, B, C, D, and the control group. SPSS software was used for this analysis. In this study, the significance setting was $p < 0.05$.

3. Results

3.1. *Gravity frequency change in time*

The gravity frequency of EEG is an important factor, which has a strong correlation with the excitability of the brain. It can reflect the transition of EEG power spectrum and indicate the alertness level of subjects.

Figure 2(a) shows the change of the difference of gravity frequency between the experimental group and the control group in time. For all four experimental groups, the difference of the gravity frequency increased first and then reached stable state in time. It is shown that the difference between the experimental group with 0 Hz flicker frequency and the control group is the smallest. The higher the stimulation frequency, the larger the difference between the experimental group and the control group on gravity frequency. The difference of the gravity frequency is about (1.12 ± 0.55) Hz in the experimental group with 0 Hz flicker frequency, (1.59 ± 0.69) Hz in the experimental group with 5 Hz flicker frequency, (1.98 ± 0.80) Hz in the experimental group with 10 Hz flicker frequency, and (2.36 ± 0.86) Hz in the experimental group with 20 Hz flicker frequency ($F = 15.653$, $P < 0.001$).

Figure 2(b) shows comparisons between flicker frequency and the difference of gravity frequency with a regression line, a correlation coefficient and a significant level. The result shows that flicker frequency and the difference of gravity frequency has a strong linear correlation.

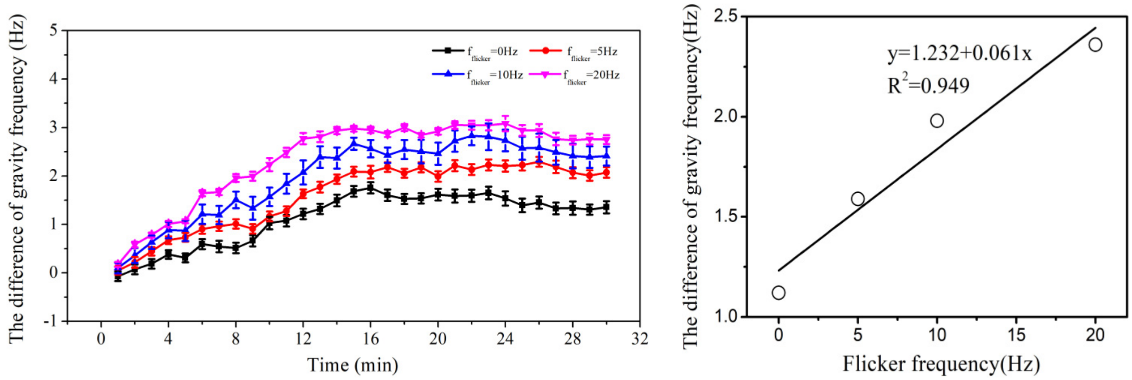


Fig. 2. Gravity frequency change in time. (a) shows the difference of gravity frequency change in time; (b) shows linear regression relationship between flicker frequency and the difference of gravity frequency $p < 0.02$.

3.2. *Relative energy changes in time based on different band*

The change of the difference in the relative energy values of A, B, C, and D among four experimental groups and the control group over time is shown in Fig. 3. In Fig. 3(a), the relative energy difference of A did not change significantly in time. However, the difference of relative energy of B decreased within the time range of 0–15 min and then reached a stable state in all four experimental groups. Specifically, as the curve becomes stable, in the four experimental groups, the higher the frequency of the

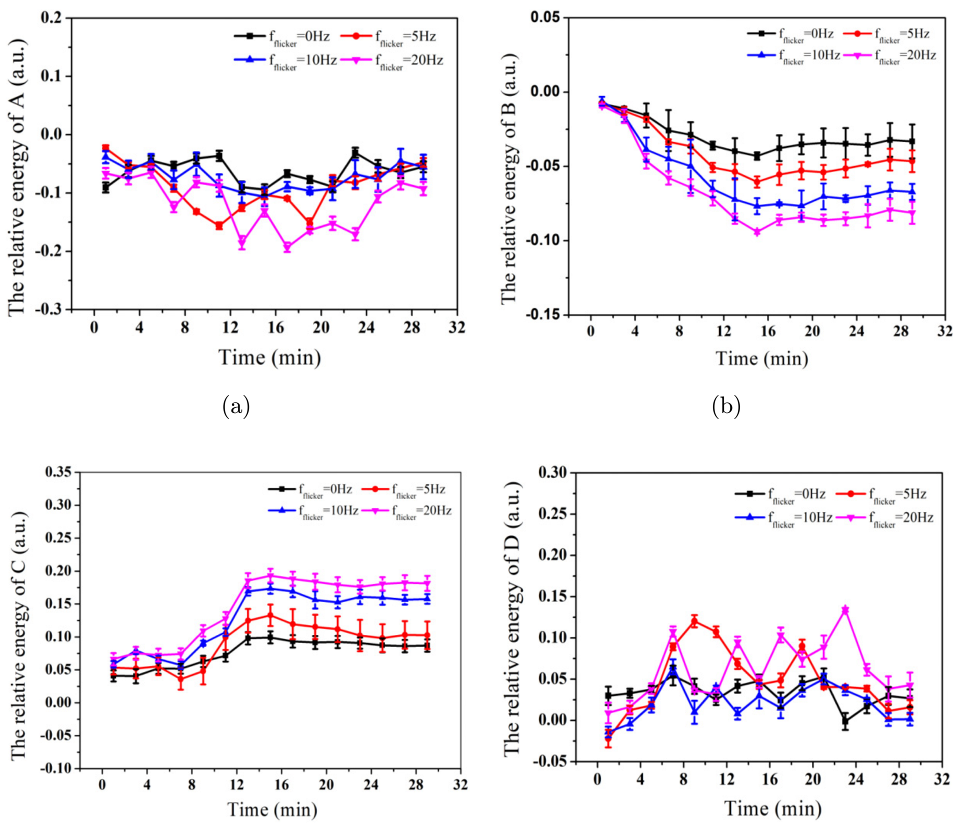


Fig. 3. The change in the difference of relative energy based on the four different frequencies: (a) The relative energy of A (0–2 Hz), (b) The relative energy of B (4–6 Hz), (c) The relative energy of C (9–11 Hz), and (d) The relative energy of D (19–21 Hz).

stimulus, the larger the change of the difference relative energy of B (the relative energy of the group of 0 Hz (-0.0289 ± 0.01073), the group of 5 Hz (-0.0415 ± 0.0135), the group of 10 Hz(-0.05703 ± 0.0224), the group of 20 Hz (-0.06777 ± 0.01787), $F = 9.488$, $P < 0.02$). Figure 3(c) shows the change of the relative energy difference of C in time. All the curves in the four groups of experiments have the trend of first rising and then reaching a steady state. After 13 min, they tend to be stable. The curve in the experimental group with 0 Hz flicker frequency is at the bottom, and the curve in the experimental group with 20 Hz flicker frequency is at the top in Fig. 3(c). The higher the flicker frequency is, the more distinct this trend is (the relative energy of the group of 0 Hz (0.0755 ± 0.02165), the group of 5 Hz (0.0894 ± 0.0322), the group of 10 Hz (0.1255 ± 0.0459), the group of 20 Hz (0.1424 ± 0.0518), $F = 8.487$, $p < 0.001$). There is a certain time delay in the brain response to NIR light stimulation due to that the response is a process of gradual change. Therefore, there will be a first rise/fall change. When a certain time threshold is reached, the brain activity will be in a stable state. Figure 3(d) shows the change of relative energy difference of D in time, but the difference curves in the five groups showed no consistent patterns.

Figure 4 shows comparisons between flicker frequency and the relative energy with a regression line. Figure 4(a) shows the relative energy of B, Fig. 4(b) shows the relative energy of C, a correlation coefficient and a significant level. The result shows that flicker frequency and the relative energy B/C have a strong linear correlation.

3.3. *Sample entropy brain mapping*

Figure 5 shows the brain topography of the electroencephalogram sample entropy. Figure 5(a) shows the initial value of sample entropy of EEG signals in the five groups, and Fig. 5(b) shows the value of sample entropy of EEG signals in the five groups at the end of the experiment. The red color indicates high activity and the blue color indicates low activity. Moreover, Fig. 5(c) depicts the difference of the entropy value of EEG samples between values at the end and the beginning of the experiment. The large differences are indicated by the red-shaded areas and there are no significant differences in blue-shaded areas. The difference in the control group was relatively small and the change was not significant. Figure 5(d) shows the sample entropy difference in significant electrodes, it is shown that the stimulation mainly caused the changes in the prefrontal lobes, and the activation was more distinct with the increase of stimulation frequency. The most obvious change was in the experimental group with a stimulation frequency of 20 Hz.

Table 1 shows the electrodes in which the entropy of EEG signals has significant differences. As shown in Table 1, the electrodes with significant differences are mainly concentrated in the prefrontal region, indicating that near-infrared light may activate the brain region near the stimulation region.

4. Discussion

In this study, we investigated whether and how NIR LED light stimulation with varying frequencies

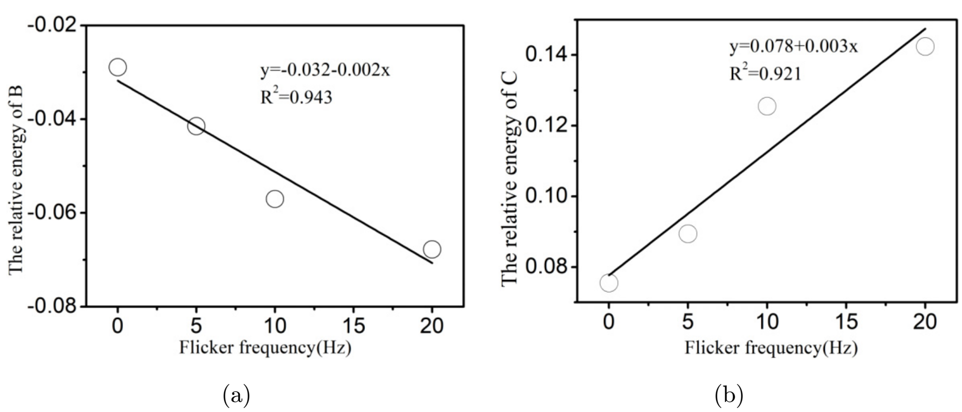


Fig. 4. Linear regression relationship between flicker frequency and the relative energy. (a) Shows the relative energy of B. (b) Shows the relative energy of C, $P < 0.01$.

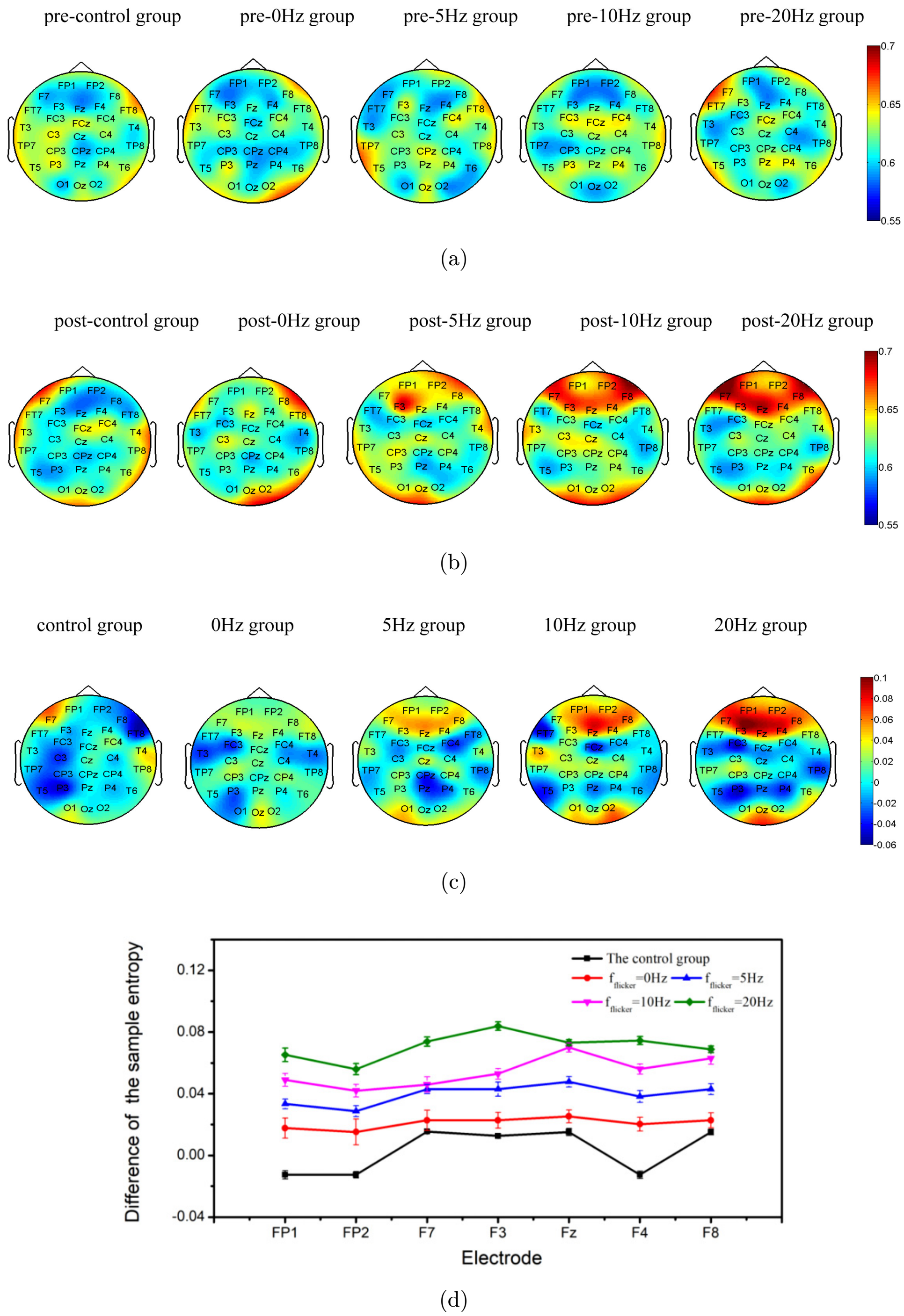


Fig. 5. Brain topography of electroencephalogram ample entropy. (a) and (b) Show control group, 0 Hz group, 5 Hz group, 10 Hz group, 20 Hz group activities between pre- and post-task of NIR LEDs stimulation. The red color indicates high activity and the blue color indicates low activity. (c) Depicts the difference between pre- and post-values. The large differences are indicated by the red-shaded areas and there are no significant differences in blue-shaded areas. (d) The sample entropy difference in significant electrodes

affect brain activity in the forehead of healthy people. Our results indicate that with the increase of stimulation frequency of the NIR LED light, the extent of the activation of brain activities in the stimulated areas is more distinct. This study may improve the design for the future PMB treatment. Our experiments showed that the change in gravity frequency is significantly different between the experimental groups and the control. Gravity frequency is larger in experimental groups compared to

Table 1. One-way ANOVA result for sample entropy.

Position	P (Sample entropy)	Position	P (Sample entropy)
Fp1	0.001	F7	0.018
Fp2	0.001	F8	0.036
F3	0.016	Fz	0.030
F4	0.025		

Note: Significant at $p < 0.05$.

that in the control. The larger the stimulation frequency, the larger the extent of the increase of GF. The amplitude of some relative energy (e.g. alpha waves) increased, while that of others (e.g., theta waves) decreased remarkably in the experimental groups, and the extent of increase/decrease is larger at higher stimulation frequency, compared to those of the control. Sample entropy of electrodes in the frontal areas were much larger than those in other brain areas. Our results indicated that NIR LED light may have an acute effect on the brain activity in certain people. With the increase of flicker frequency, the effect become more noticeable. When the stimulation was focused on the prefrontal region, the activation was mostly located in the forehead, the rest of the brain was not much affected. Whether similar phenomenon exists in the rest regions of the brain is unknown yet, which awaits more studies in the future to clarify this issue. Findings from this study showed great potential for developing nondestructive phototherapy for brain disorders.

Recent human studies have shown that NIR LED light can promote the healing of chronic craniocerebral injury.[25] The mechanisms behind it is not clear yet. But studies have shown that light in the NIR LED band can affect the permeability of the membrane and ion transport by affecting the calcium channel. Calcium ion fluctuations are prevalent in synaptic and extracellular processes, which can be reflected indirectly by detecting EEG signals.[26] Recently, a few researchers found some correlation between NIR LED stimulation and the EEG signals change. For example, Spera *et al.*[4] studied the effects of different doses of NIR LED stimulation on EEG oscillations, and showed that NIR LED had a dose-dependent effect on the activity of gamma and beta neurons in the brain. These work has soundly shown that NIR LED light stimulation affects brain activities.

tPBM has significant value in enhancing cognitive function and other brain degenerative diseases. Using an 810 nm NIR light pulse at 40 Hz, Zomorrodi *et al.*[12] found that a single session of tPBM significantly increased the power of the subjects' alpha, beta and gamma frequencies and decreased the power of delta and theta frequencies in the resting state. Jahan *et al.*[27] studied EEG signals recorded before and after NIR LED stimulation and found that NIR LED stimulation shortens people's the reaction time. Zomorrodi *et al.*[28] dispensed tPBM into the default mode network (DMN) in the form of pulsed NIR light and tested its influence on the power spectral density. The results showed that active stimulation produced a significant power increase in the alpha, beta, and gamma bands compared to sham. The results of these studies provide evidence for the regulation of brain function by tPBM.

To further understand how NIR LED light stimulation affects the brain activities, in this study, we investigated how different stimulation frequencies of the NIR LEDs light will affect the brain activities and how these effects change during the stimulation process through measuring and analyzing EEG signals, especially the several important features inside the signal, such as the gravity frequency and the relative energy change.

The gravity frequency of EEG signals could serve as an important indicator for certain brain activity and thus may serve as an important evaluation index for the outcomes of brain phototherapy. The gravity frequency reflects the transition of EEG power spectrum, which indicates the subjects' alertness level. At the same time, it can not only characterize the larger frequency components of the signal, but also reflect the migration of the center of gravity of the EEG under different conditions.[29] Through analyzing EEG signals, Yong *et al.*[22] found a positive correlation between gravity frequency and the brain fatigue level, in which the lower the gravity frequency, the higher level of the fatigue the brain has. These studies have shown that gravity frequency of EEG signal can be used as an index for evaluation brain function during/after phototherapy. In our study, we investigated effects of different stimulation frequency on the gravity frequency to assess certain brain activities. Our results show that with the increase of the stimulation frequency, the EEG signal was significantly intensified, suggesting that increasing the stimulation frequency of NIR LED light could result in stronger activation of certain brain activities. Figure 2(b) shows that flicker frequency and the difference of gravity frequency has a strong linear correlation. The result indicates that within a certain frequency range, increasing the stimulation frequency may enhance the activity level and alertness of the brain.

The other important parameter we choose to analyze in this study is the relative energy changes of EEG signals, as it is another important indicator of the brain activities. The relative energy of alpha is an important parameter of EEG signals. It has been found that alpha waves are associated with memory, for example, Klimesch *et al.*[30] reported that people with better memory had higher alpha frequencies. In addition, Compton *et al.*[31] showed that the relative energy of alpha band increases when the person is in a wandering state which helps restore the memory function of the brain. In our study, we found that NIR LED light stimulation increases the relative energy of alpha wave, and the increase is stimulation frequency dependent, that is, the higher the stimulation frequency, the larger the increase of the relative energy of alpha wave. Figure 4(b) shows that flicker frequency and the relative energy C has a strong linear positive correlation. Our results suggest that NIR LEDs light stimulation may be beneficial to the improvement of memory, and higher stimulation frequency may have better effects on improving the brain memory function. Our findings on the relative energy of B that first decreased with time and then remained unchanged, Fig. 4(a) shows that flicker frequency and the relative energy C has a strong linear negative correlation. The results indicate that with the increase of stimulation frequency, the decline of brain activity slows down. The difference of beta waves is not obvious under the stimulation of several groups of experiments, mainly because beta waves will be inhibited in the closed eyes and rest state.

Since several works require continuous alertness like efficient driving, learning, etc. maintaining and improving alertness is is a crucial challenge for researchers.The increase of fast wave components of EEG signals, the gravity frequency and sample entropy complexity are important indicators to evaluate the improvement of brain alertness.[32] Our experiments showed that gravity frequency is larger in experimental groups compared to that in the control. The larger the stimulation frequency, the larger the extent of the increase of gravity frequency. The relative energy of alpha increased remarkably in the experimental groups, and the extent of increase is larger at higher stimulation frequency, compared to those of the control. Sample entropy of electrodes in the frontal areas in the experimental groups were much larger than those in the control. Our results indicated that NIR LEDs light may improve brain alertness in certain people. With the increase of flicker frequency, the effect become more noticeable.

In addition, the sample entropies collected from all channels distributed all over the head were observed, and it was found that the prefrontal and occipital lobes were mostly responsive to the stimulation, probably because the stimulation was mainly applied to this area. These results may indicate that the response may be stimulation location-dependent, but more studies are needed to confirm this phenomenon. In the next study, the phototherapy evaluation of multiple brain areas could be carried out. Studies have shown that removing the prefrontal lobe of animals can lead to serious behavioral abnormalities and behavioral aimlessness.[33] The prefrontal lobe is closely related to many cognitive skills and sophisticated thinking activities. It also participates in the final processing and storage of various information. Based on this, the forehead was selected in NIR LED radiation experiment group and significant results have been achieved. Fred *et al.*[34] found that 20-minute stimulation of NIR LED light to the temporal, occipital and frontal lobes may have an acute effect on reaction time in a subset of subjects with doses different from that used in this paper. In our future study, multiple brain regions radiation with different frequencies will be conducted. In this study, NIR LED phototherapy appeared to be safe, with no adverse events reported. Therefore, it is believed that the possibility of photo-chemically induced damage is low.

The LED diodes have low thermal effect, however multiple diodes in a closed box may increase the temperature of the forehead, and changes in temperature may affect the accuracy of the experimental results. In our study,forehead temperature was monitored throughout the experiment, and remained stable in both the control group and the experimental groups, thus, we think the effect of heat generated by the NIR LED light during the 30 min and resultant temperature rise is minor and would not significantly affect the accuracy of our data and results.

Whereas, there are still several limitations. First, the sample number is not large enough, only 50 subjects were used in the study, thus the statistical reliability would be limited by the number. With the increase of the database, we will collect more and more cases and improve the reliability of the measurement. Second, we only evaluated three EEG parameters and examined the potential efficiency of these features. To improve the accuracy of diagnosis, more additional features may need to be included. In this paper, we only studied the effects of four NIR LED flicker frequencies on EEG signals, and more stimulus frequencies can be considered in future studies.

5. Conclusion

Considering the pending questions in clinical PBM applications, the study investigated whether and how NIR LEDs light stimulation with varying frequencies affect brain activity in the forehead of healthy people by analyzing the measured EEG signals during the stimulation process. The conclusions are as follows:

(i) The EEG signals of the control group and the experimental groups (0 Hz, 5 Hz, 10 Hz, and 20 Hz) were significantly different, indicating the effectiveness of low-energy NIR LED phototherapy. The gravity frequency analysis and relative energy analysis were used to evaluate the effects of phototherapy.

(ii) The EEG signals after NIR LED light stimulation are different among different groups. With the increase of NIR LED light frequency, the gravity frequency increased more quickly, indicating that brain activity would be more active, while the increase of relative energy of C is larger, indicating that higher frequency of NIR LED light stimulation may have better improvement effects in brain memory.

(iii) Based on the EEG sample entropy analysis of all channels over the whole brain, the EEG signals in different channels at the same frequency has significant differences. The study showed that the main activation site of NIR LED light was in the frontal region, and the activation became more remarkable with the increase of stimulation frequency. The change of sample entropy of EEG signals is also stimulation frequency dependent, which also supports the effectiveness of NIR LED light radiation. These results may provide new possibilities for the improvement of phototherapy technology.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgments

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