Advances in Bioresearch
Adv. Biores., Vol 10 (2) March 2019: 108-119
©2019 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.10.2.108119

Advances
in
Bioresearch

ORIGINAL ARTICLE

Innovative method in Neurotherapy, neurology and psychiatry; Investigation of Transcranial laser stimulation (TLS) on EEG rhythms

[1]Yasaman Zandi Mehran*, [2]Michael Weber
[1]Department of Electronic, College of Electrical Engineering, Yadegar – e -Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University, Tehran, Iran.
Department of Biomedical Engineering, Science and Research Branch, Tehran, Iran.
Email: zandi@srbiau.ac.ir
[2]Medical Director Rothenburg Congress AGTCM 2013-2016
President of European TCM Laser Academy
University Witten – Herdecke, Germany

ABSTRACT

The use of transcranial direct current stimulation (tDCS), and transcranial magnetic stimulation (TMS) in many clinical indications in neurology, and psychiatry is well established. Parallel to these researches other scientific studies have shown clinical effects with strong evidences by the use of local Low Level Laser (LLL) in similar clinical conditions. In this paper, we investigated similarities and differences of these methods. We investigated the efficacy of LLL on electroencephalogram (EEG) in a double-blind sham control study. Forty healthy male volunteers aged between 22 and 34 years were randomly assigned to either experiment or sham group. In the present study, subjects in experiment group were exposed to 10Hz at F3 by a LLL radiation, using 1J, 904nm, 300W, pulsed (pulse width 200nsec), approximate time of exposure is 30 minutes in one session. The EEG was recorded before exposure, 2 minutes exactly after exposure, 30 minutes and 90 minutesafter exposure. In this paper, we report the results of EEG changes exactly after exposure. Results indicate that in many regions of the brain, the theta, and alpha EEG rhythms variations in exposed group changed significant in comparison of sham group in both frontal and central regions ($P < 0.05$) after exposure. Results suggest that compared to sham exposure; some of Local LLL effects significantly persists approximately 90 minutes after exposure. It seems that by LLL radiation, we can change EEG rhythms, and maybe affect brain activities. More studies are needed for finding a conclusion of laser effects on brain, and its functions.

Keywords: TLS, EEG rhythms, LLL therapy

Received 15.10.2018 Revised 21.10.2018 Accepted 13.12.2018

How to cite this article:
Yasaman Zandi Mehran, Michael Weber. Innovative method in Neurotherapy, neurology and psychiatry; Investigation of Transcranial laser stimulation (TLS) on EEG rhythms. Adv. Biores. Vol 10 [2] March 2019: 108-119

INTRODUCTION

The biological effects of weak electrical currents on brain and neuronal function were already first described decades ago. There is a rapid increase of brain stimulation instruments to influence the various brain functions. Brain stimulation has been suggested by different techniques [31, 32, 41, 42, 16, 37, 29]. These methods and techniques are used in brain stimulation which is applied in variety of clinical conditions such as neurotherapy, neuropsychiatric treatments and brain performance improvement [10, 28, 27, 41]. Magnetic, Electromagnetic and Electrical stimulators are included in this kind of stimulation; such as, TMS (Transcranial Magnetic Stimulation), rTMS (repetitive transcranial magnetic stimulation), ELF (extremely low frequency magnetic field), tDCS (transcranial direct current stimulation), tACS (transcranial alternative current stimulation), tPCS (transcranial pulse current stimulation), tRNS (transcranial random noise stimulation), HD-tDCS (high definition tDCS), ECT (electroconvulsive therapy) etc.

Two these methods are in favor of neuro-therapists nowadays: tDCS, and rTMS.

There is a fundamental difference between TMS and tDCS. tDCS is a simple technique of noninvasive brain stimulation in which a weak DC current is applied into the brain for several minutes resulting in a polarity-dependent modulation of brain activity. Traditional form of tDCS will influence the cortex, while by magnetic stimulation subcortical regions are affected. Both of these noninvasive methods produce the polarization in brain cells to attain stimulation ability.

Brain function is affected differently in anode and cathode in tDCS method, where brain cortex acts as a conductor and therefore electrical current is directed from superficial layer of neural tissue. In tDCS there is a modulation without action potential production whereas TMS causes action potential. TMS has two basic mechanisms which are neuro-modulating and stimulation.

The interesting point in electrical current brain stimulation methods is that when one side of the brain is stimulated, positive effects appeared on the opposite side. The advantage of tDCS over TMS is that in tDCS the both sides of brain can be stimulated simultaneously, whereas TMS can be applied only on one side.

In general protocols based on the suppressive of a wave have better performance than stimulation-based protocols. For example instead of amplifying alpha band in one zone in can be suppressed in other zones.

To conclude on all studies published so far they postulate these methods as an efficient technique for brain stimulation in many indications such as depression, sleep disorder, migraine, cognitive improvement, fibromyalgia, anxiety, addiction, etc.

But unfortunately, usage of the tDCS is harder than the transcranial laser or transcranial magnetic stimulation. The current amplitude in tDCS is 1 to 4 mA, since people have different skin resistance the resultant current is different. The effects of the traditional and cheaper version of the tDCS is most of the time on the cortical regions, and we can hopefully respect to have effects on the brain network. The TMS is more respectful, but unfortunately a precise version is more expensive, and if the precision change, it could be harmful.

On the other side, over more than five decades clinical studies and research on LLL has been performed [9]. LLL therapy is an established method in cosmetic and dermatology as well as in pain treatment [1, 3]. There are many meta-analyses about the mechanism and effects of LLL therapy on wound healing, pain reduction, etc [13, 14, 17, 38, 36, 9,5, 6].

Laser is the abbreviation for Light Amplification by Stimulated Emission of Radiation, reflecting a unique form of electromagnetic radiation [19]. The physical concept of stimulated emission was proposed by Einstein in 1916, and after a half of century later on, in 1960 Maiman was the first to create a handhold laser by using a synthetic ruby crystal. The laser has a high degree of spatial and temporal coherence that makes this light source unique. It has many parameters, such as wavelength, diode driving (continuous, modulated, or pulsed), power, etc. Mester from Hungary was the first scientist to describe the cellular effects of LLL. LLL is a type of laser that has photobiomodualtion (PBM) effect in all type of cells in body, esp. to mitochondria. By changing of these parameters you can manage the target, depth of penetration, and the type of stimulation (excitatory or inhibitory) effects [18].

In recent years, LLL therapy has been in the research focuses all over the world: stroke, traumatic brain injury, neuro-rehabilitation, Parkinson disease, Alzheimer, and even peripheral nerve regeneration etc [21, 22, 39, 40, 20, 35]. They are striking scientific result in medicine about excellent effects of LLL therapy in myocardial infarction, and degenerative or traumatic brain disorders, and othersevere chronic disease [30-34, 7, 8].

LLL therapy is targeting mitochondria: main target of LLL are the photoreceptors of the respiratory chain within the inner mitochondrial membrane, especially Cytochrome – C – Oxidase (CCO / Complex IV). The cell-physiological effects of LLL therapy consists many aspects such as increase adenosine triphosphate (ATP), temporary increase in reactive oxygen species (ROS), changes in intracellular calcium, and release of nitric oxide (NO), activating of Heat Shock Proteins (HSP) etc. By inducing signal processes and activation of transcription factors gen-expression of many protective, anti-apoptotic, anti-oxidant, and pro-proliferation gene products is achieved [23, 24, 33, 34, 18, 19].

Animal studies as well as human clinical trials of LLLTconfirm strongly the efficiency of LLL in neuro-therapy. There is no doubt that near infra-red light (NIR) can penetrate the scull bone and can affect cortical or subcortical structures. Many specialists of PBM believe that LLL can replace chemical substances in many clinical settings such as neurological disorders. However, there is little information in the literature about direct influence on brain function [12, 20, 39, 40].

The aim of this study is a simple and preliminary investigation of LLL on brain activity by evaluation of EEG signal.EEG is a well-known and widely used technique for assessment of brain activity. It has been suggested that power spectrum might be used as a tool for visualizing the effects of a specific stimulation protocol as well as for evaluating the physiological outcome of the stimulation. In this paper, evaluation of brain activity, QEEG (Quantitative EEG) was performed before and after laser radiation: before exposure, 2 minutes exactly after exposure, 30 minutes and 90 minutes after exposure.

All studies confirmed that QEEG evaluation is related with brain function. For example, Alpha waves are neural oscillations in the frequency range of 8-13Hz and are associated with calmness and consciousness, waves predominantly originate from the occipital lobe during wakeful relaxation with closed eyes. Alpha band is observed here while a prob and is lying. Alpha band is recorded while a patient is conscious and reactive to its environment (alertness). So in this case, the subject is relaxed and ready to react to surroundings simultaneously. Although, Alpha band increment has different meaning in different regions, e.g. the increment of alpha band in frontal region shows depression or ADHD. Alpha band increment in occipital region shows sleep disorders. - In this study, we evaluated the LLL result on QEEG bands before and after of LLL radiation in comparison with sham group.

PROTOCOL INVESTIGATION

In the literature there are many studies indicating that that modulated LLL radiation (frequency)is more efficient than continuous radiation (cw) [1, 32, 36, 26]. However, the clinical data are still incomplete. There are some researches on effects of LLL radiation when the frequency is applied from 10Hz until 10kHz in comparison with cw. The result indicates that 10 Hz is significantly more effective. Also they are usage of 10 Hz tACS, and rTMS is significantly efficient on brain activity. This frequency is near mean of alpha band in brain activity.

On the other side, some studies indicate that electromagnetic field matches the angular frequency and resonance phenomena investigations. These researches believe that at some frequencies, LLL causes reinforcement of the same frequency as the exposure field’s one. These studies investigate the effects of very weak alternating magnetic and electromagnetic fields upon living organisms and related variations in the ion concentrations within the cells when the frequency of the applied field matches the angular frequency. This phenomenon is called resonance mechanism by which biological systems become sensitive to small static and resonating fields and existence of a resonating effect on ions. These studies are applicable for both magnetic and LLL effects. De Ninno *et al.* [11] described that Larmor frequency of the most of the ions involved, lies between 10 and 50 Hz. The interaction of spin-correlated radical pairs with magnetic fields confirms magnetic effect to account for the Larmor frequency coupling. This study describes that Larmor frequency of the Fe^{2+} ion and Cu^{2+} are 17 Hz and 15 Hz respectively. The Larmor precession provides a mechanism by which biological systems become sensitive to small static and resonating magnetic fields [13, 14]. The weak magnetic fields strongly decrease an enzyme catalytic activity that affects the modified availability of Ca^{2+} due to magnetic field. Also there are many studies that indicate we can effect on cells by laser frequency extremely selective. Although basically three electrical, magnetic, and electromagnetic fields are intrinsically different in origin, but they are correlated to each other, and there is some similar concept like resonance in all three fields.

Studies on penetration depth of LLL proved in a pulse laser diode, 904nm, the penetration depth is near 5 centimeter, and can penetrate through skull bone easily. The beam of laser is narrow. By radiating with a narrow beam of laser, we can reduce the area of exposure. LLL can be used as a device for brain stimulation and postulated here even for brain activity control [18, 19].

After clinical use of LLL for almost six decades, there are no reported side effect for LLL [19]. However, there are some studies published denying a beneficial clinical effect. In most of these studies the protocol was basically incorrect; sometimes the dose or power of LLL is extremely lower than standard, and sometimes the wavelength was chosen incorrect, e.g. they used continuous 630nm for radiation, and the penetration depth if this wavelength in this status is less than 1 cm.

There is a vast of studies proving the bio-stimulatory effects of LLL, and there is good evidence that LLL exposure has crucial effects on cell and nerves, brain and brain activity. Although no intensive and systematic side effect have been described in the literature, one of the aims of this study is evaluation and estimating of a deep laser radiation so that have considerable influence on cerebral signals which can be used in protocols to treat some psychological and neurological diseases without adverse effects. Based on WALT recommendation for dose (https://waltza.co.za) for Laser class 3B, 904 nm GaAs Lasers, a minimum of 1 Joules per point or area (cm^2) is needed. We used this minimum for total local dose. If this dose proves to be efficient, we postulate that 4 joules is even more efficient.

For choosing a place on the brain, and examine laser radiation, we found that many different networks do exist in the brain 4, 7], i.e. a regulation of affect may be imposed by the prefrontal cortex (PFC) and its efferent to the amygdala. By the intracranial network hippocampal and amygdala inputs interact with the prefrontal cortical inputs to the nucleus accumbens,a region in the basal forebrain rostral to the preoptic area of the hypothalamus.

Also based on the known networks in the brain, the DLPFC has connections with the orbitofrontal cortex, thalamus, dorsal caudate nucleus ,hippocampus and primary and secondary association areas of neocortex (including posterior temporal, parietal, and occipital areas).There are many papers that used tDCS, and rTMS on DLPFC especially by stimulating F3 (based on 10-20 international system) for having an effect on this region, or on the other parts of brain via networks.

The aim of this study is evaluating the EEG changes in all regions of brain, by 10Hz radiation at F3 (left side of Frontal) by a LLL, 1J, 904nm, 300W, pulsed (pulse width 200nsec), approximate time of exposure is 30 minutes per session. – The EEG represents the dynamics of the entire brain as a unified system. Each EEG is commonly decomposed into EEG sub-bands: delta (0-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (30-60 Hz). We investigated all of the variation of these sub-bands after LLL radiation with minimum dose, to evaluate the transcranial laser stimulation method on a brain activity.

MATERIAL AND METHODS

Sample Volume Estimation

The sample volume should be determined before each experiment. With regard to most papers and studies investigating tDCS, and rTMS effects on EEG signals, the number of subjects varies around 20. In the sham group, the number of samples is the same. This group defined for better comparison. As a first step, it is necessary to utilize sample volume determination in the experiment based on error types and targets. If a test is used for reviewing differences and error types, and α and β are considered, then sample volume can be achieved by:

$$(1) \qquad n = \frac{(Z_\alpha + Z_\beta)^2 \times (SD_1 + SD_2)^2}{(\mu_1 + \mu_2)^2}$$

Where SD is standard deviation of each experiment and μ is the mean. This equation is used when t-test is used for comparison of two groups and α and β are considered too. If α is 0.05 then $Z_\alpha = 1.96$. β is considered 20% and power test is 80% (Z_β is 0.84). If we consider expected variations in EEG rhythms of pilot study then n is calculated approximately. According to sample volume estimation, literature, and study goals, 8 are sufficient for the sham group and the experimental group, separately. Based on previous studies, 20 subjects are required for the exposed group and 20 for the sham group.

Subjects

Forty heathy male volunteers aged between 22 and 34 years (mean age of 27.39±1.04 years) in good physical and mental health were attend in the study properly and ethically informed about LLL exposure and experiment. The body mass index (BMI) of all volunteers was in normal range. They did not receive any previous stimulative treatment method on the brain. Criteria for exclusion were checked by biological examination and a health questionnaire as follow: psychiatric diagnosis, diabetes, CNS disorders, epilepsy, alcohol intake, drug intake, smoking, a cerebral metallic implant. None of them had previously taken part in studies involving laser exposure and didn't have any surgery. All subjects were asked to refrain from drinking coffee and even tea 12 hours before attending the experiment.

Study Design

This study was conducted as a sham-controlled, double blind study in which the laser probe was placed over the left DLPFC according to F3 (10-20 EEG system). In the sham group the laser device was putted exactly on the same place. After pressing start key, the beep was turned on in both groups, but we have a real radiation in experiment group, and no radiation in sham group. There is a LED that is always on this probe (after turning on the power supply of the laser probe). By use of nonvisible infrared laser (wavelength 904nm, Reimers & Janssen GmbH RJ-LASER, cluster prober, RJ laser, Germany) it was possible to create a real double blinded protocol. All stimulation sessions were carried out by the same person. All patients underwent one session for 30 minutes/day without a break between. No electromagnetic device like cell phone is near the volunteer in the room, and a participant is seated in middle of room. The window of room was covered by aluminum foil. The light of the room was extremely low.

Data Recording and Analysis

For recording of EEGa 19 electro-cap was used(electrodes were placed on Fp1/ Fp2, F3/F4, C3/C4,P3/P4,O1/O2,F7/F8,T3/T4,T5,T6,Fz,Cz,Pz by 10- 20 system and reference was A1+ A2 electrode). The electrodes impedance was under 5kΩ for all electrodes while all of the subjects were resting and closing their eyes, the EEG has recorded in 10 minutes at an acoustic room with Mitsar system. Data were recorded with 250Hz sample rate, and with high frequency filter of 50 Hz and low frequency filter of 0.3 and 50 Hz of notch filter.

The EEG frequency range has been divided into these categories: delta (0-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), and beta (13-20 Hz). Frequency analysis of the 19 channel EEG was subsequently carried out using Fast Fourier Transform (FFT) to estimate power spectrum. After averaging a power spectrum of segments and obtaining a *mean relative* power spectrum in each status of all the subjects before and after radiation, based on the contribution of the EEG rhythms. Statistical analysis is used to find the most discriminative EEG rhythms in the two times of attendance, PRE, andPOST1 in distinguishing the subjects exposed to LLL from those who really weren't (sham). IBM SPSS Statistics ver.21 software was used to serve this aim. An alpha level of 0.05 was used for all the statistical tests. A comparison of the exposure and sham groups was carried out separately. For export of EEG sub-bands we used MATLAB (R2012a, Ver. 7.17.0.739).Due to the windowing of a signal that causes changes in its spectrum and frequency specification and to develop non-zero values at frequencies other than its real spectrum, the 256×2 samples hanning (2 second with 256Hz sampling rate) window are used to multiply the EEGs. For 2 minutes of recording the signal is divided into 120 parts. The mean of exported EEG bands are done along each signal.

Procedure

The study was performed between 8.30 and 13:00. Before a session, they were requested to sign a bioethics papers confirm that they are aware about LLL radiation on the brain. They were also verified by Sina Psychiatric Institute IQ test related to Intelligence quotient. This information was a quantified index to sieve normal volunteers. For both experiment and sham groups, procedure lasts approximately2 hours. After preparing the subjects, the PRE signal was recorded for 2 minutes. Then 5 minutes later, the LLL exposure starts for 30 minutes, and exactly after that POST1 signal was recorder for 2 minutes. Then the subject take rest on the seat for 30 minutes, and after that again 2 minutes EEG was recorder (POST2). This process repeats after 60 minutes later after LLL exposure (POST3). During this time, we just want from subjects to take rest on seat. Table 1 describes the procedure in each session for the two groups.

Table 1. Description of the procedure.

Interview	Group		PRE	LLL radiation for 30 minutes	POST1	Taking rest for 30 minutes	POST2	Taking rest for 60 minutes	POST3	Talking about experiment
			2 minutes		2 minutes		2 minutes		2 minutes	
	LLL Exposure (Experiment)	Preparing for LLL radiation and EEG	EEG		EEG		EEG		EEG	
	Sham		EEG		EEG		EEG		EEG	

Statistical Analysis

After recording the EEG signals of the groups, the signals were analyzed holistically. Each volunteer as described in Table 1 consisted of two statuses, namely PRE, and POST1. Frequency analysis of the EEG was subsequently carried out using Fast Fourier Transform (FFT) to estimate power spectrum. After averaging a power spectrum of2 seconds segments of EEG signal and obtaining a mean power spectrum in each status of all the subjects, the contribution of theta and beta EEG rhythms were extracted.

Statistical analysis is used to find the most discriminative EEG rhythms in the PRE, and POST1statuses in distinguishing the subjects exposed to LLL from those who weren't. This analysis was done for recorded data exactly after exposure, and also 120 minutes after exposure.

A comparison of the exposure and sham groups was carried out separately. The Kolmogorov-Smirnov test showed that some EEG rhythms didn't belong to normal distribution, so Wilcoxon signed rank test across the statuses of PRE, and POST1 were adopted. Mann-Whitney U test was used to compare the two groups with regard to their theta, alpha, and beta mean relative power. These results are described using Mean and Standard Error (mean ± SE).

Between-group comparisons showed no significant difference between the groups at pre-testing for age, and IQ. The real mean for age in the exposed group is (28.52±3.54)and for the sham group is (26.26±4.27)years old.

RESULTS

The aim of this study was to evaluate the effects of transcranial LLL on EEGexactly after exposure and after given intervals. Based on the design, the two comparisons of PRE versus POST1 in each of the two groups are presented below.

The mean amplitude for the sub-bands of EEG rhythms (theta, alpha, and beta) in 19 channels for each of the two groups across the two statuses are shown in Fig. Figure 1untilFigure 5. In this paper, analysis was done exactly after exposure.

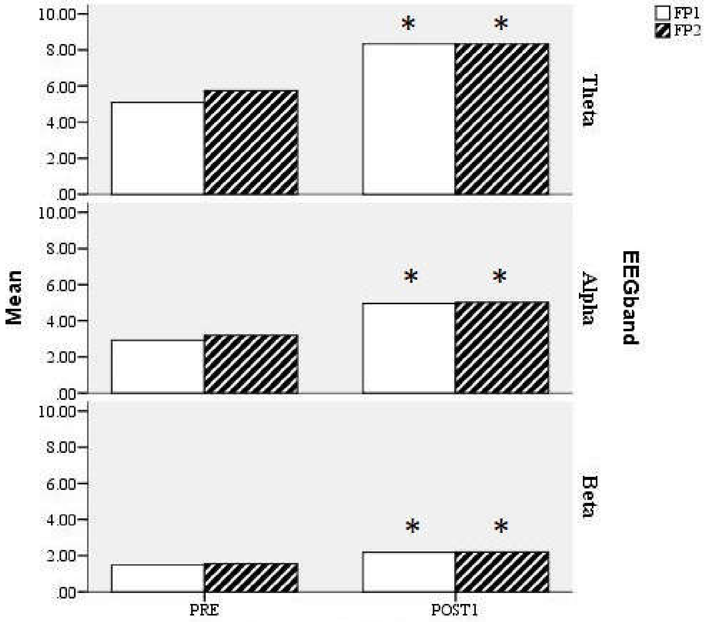

Figure 1.the Theta, alpha, and beta EEG rhythms in the PRE, and the POST1 statuses for experiment group, in FP1, and FP2 regions, the perpendicular axis is mean of amplitude of relative power of EEG.

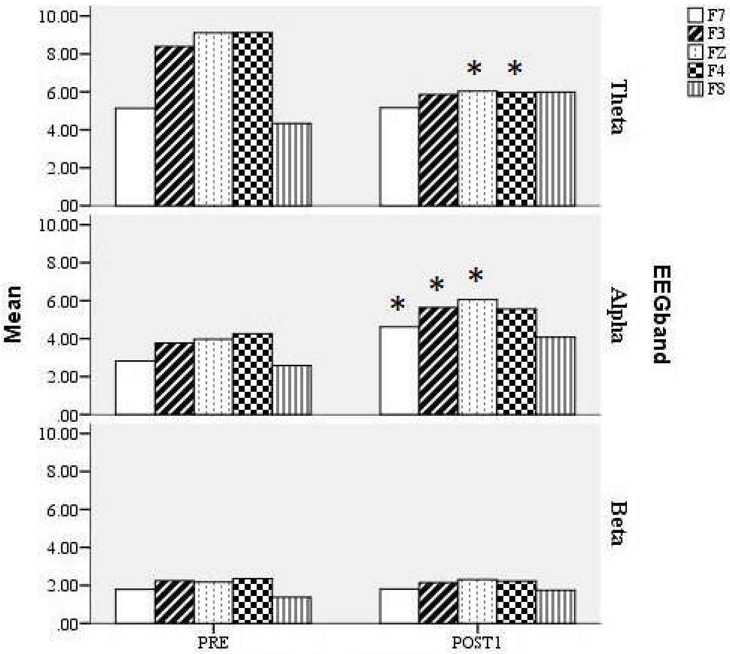

Figure 2. the Theta, alpha, and beta EEG rhythms in the PRE, and the POST1 statuses for experiment group, in Frontal region (F7, F3, FZ, F4, F8), the perpendicular axis is mean of amplitude of relative power of EEG.

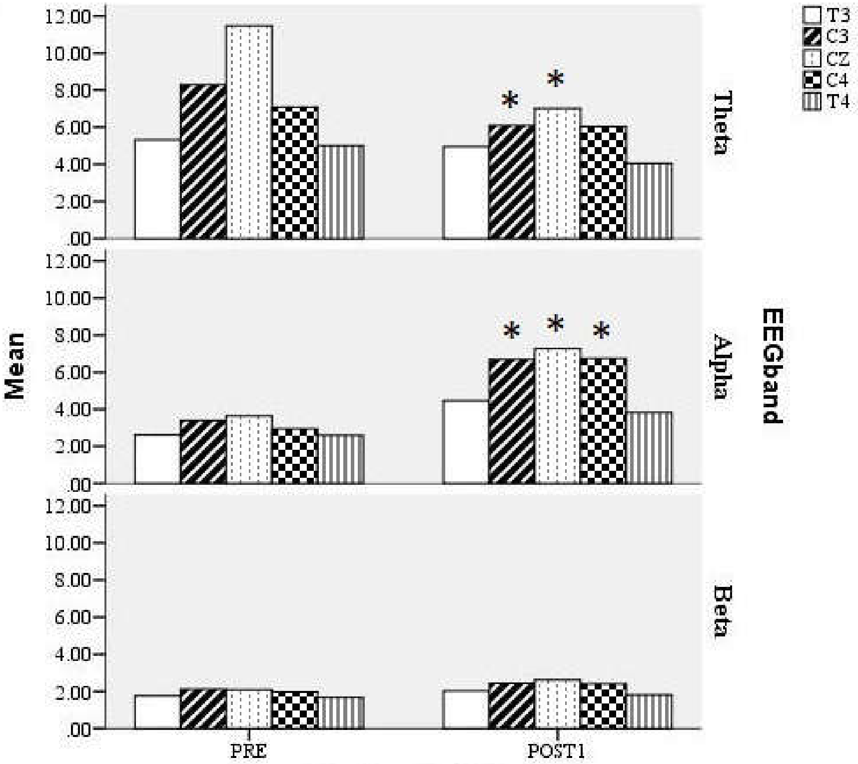

Figure 3. the Theta, alpha, and beta EEG rhythms in the PRE, and the POST1 statuses for experiment group, in T3, C3, CZ, C4, T4 regions, the perpendicular axis is mean of amplitude of relative power of EEG.

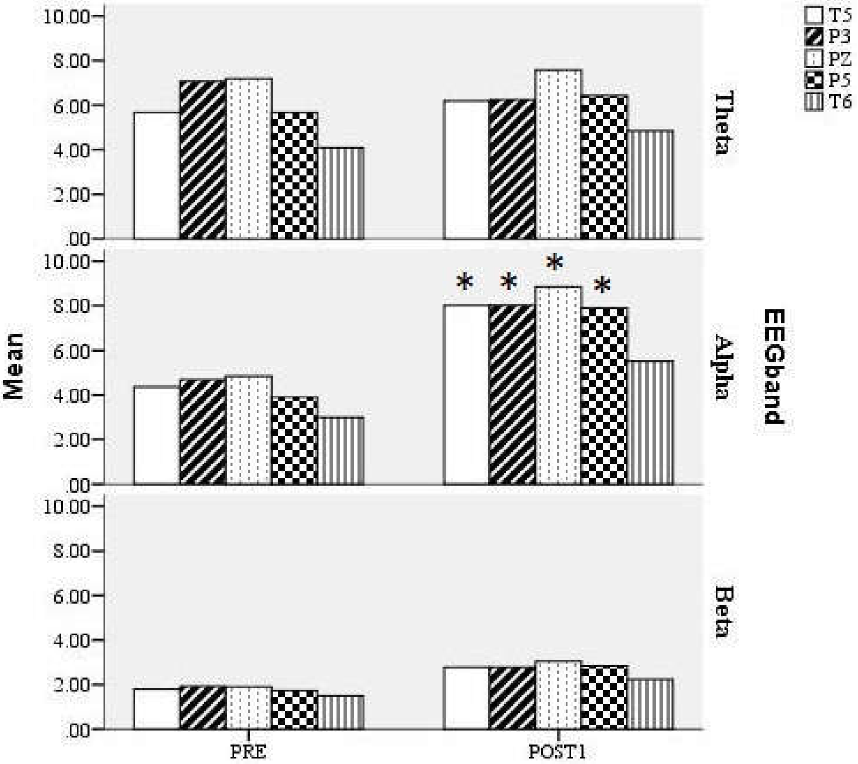

Figure 4. the Theta, alpha, and beta EEG rhythms in the PRE, and the POST1 statuses for experiment group, in T5, P3, PZ, P5, T6 regions, the perpendicular axis is mean of amplitude of relative power of EEG.

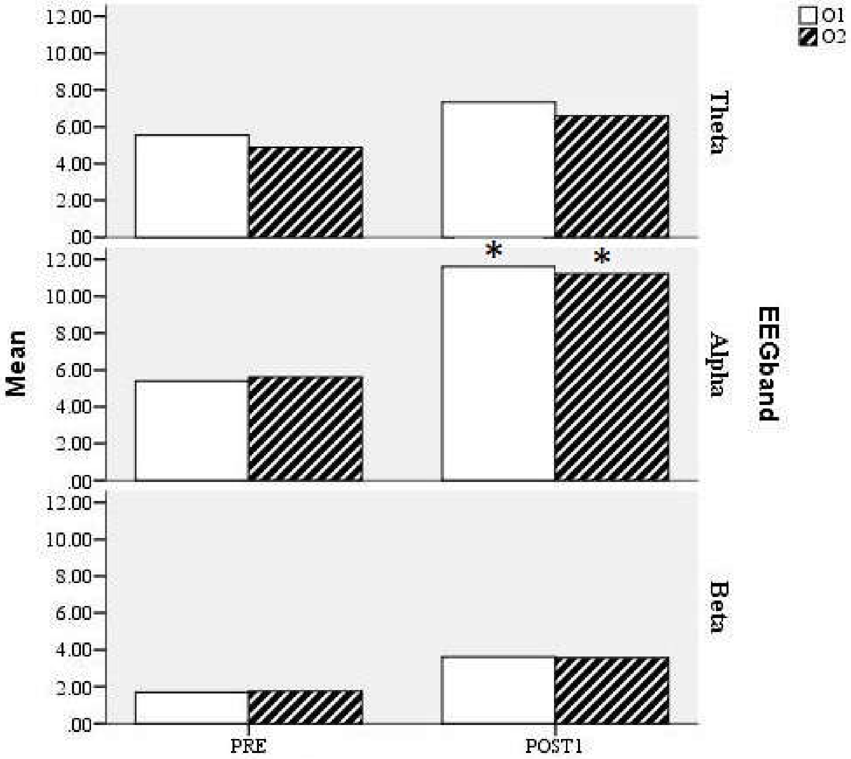

Figure 5. the Theta, alpha, and beta EEG rhythms in the PRE, and the POST1 statuses for experiment group, in O1, and O2 regions, the perpendicular axis is mean of amplitude of relative power of EEG.

As the results indicate, there was no was no significant difference in the theta, alpha, and beta EEG sub-bands in the PRE status, between the experiment and the sham groups ($p<0.05$).- Also there was no significant difference between the theta, alpha, and beta EEG sub-bands in both PRE, and POST1 statuses in sham group ($p<0.05$).

However, the theta, alpha, and beta EEG rhythms recorded from the exposed and sham groups show significant results in the PRE status in comparison with that POST1 status in experiment groups.As shown in Figure 1, all the EEG rhythms at the FP1, and FP2 changed significantly ($p<0.05$). The relative power of the theta band at FP1 at the PRE status (5.10±1.12) increased in the POST1 status (8.33±0.78) significantly ($p<0.05$). The relative power of the alpha band at FP1 at the PRE status (2.92±2.02) increased in the POST1 status (4.96±1.78) significantly ($p<0.05$). The relative power of the beta band at FP1 at the PRE status (1.48±0.12) increased in the POST1 status (2.18±0.56) significantly ($p<0.05$). Also, the relative power of the theta band at FP2 at the PRE status (5.75±0.55) increased in the POST1 status (8.33±0.32) significantly ($p<0.05$). The relative power of the alpha band at FP2 at the PRE status (3.20±1.02) increased in the POST1 status (5.02±0.98) significantly ($p<0.05$). The relative power of the beta band at FP2 at the PRE status (1.55±0.62) increased in the POST1 status (2.20±2.41) significantly ($p<0.05$)

As demonstrated in Figure 2, the alpha band of EEG rhythm increased significantly at F7 in the PRE status (2.92±1.23) in comparison to the POST1 (4.62±0.98). There was no significant change in the theta, and the beta EEG sub bands in this region.

Also, the alpha band of EEG rhythm increased significantly at F3 in the PRE status (3.77±1.93) in comparison to the POST1 (5.64±1.08). There was no significant change in the theta, and the beta EEG sub bands in this region.There was a significant decrease in the theta of the PRE status (9.11±2.17), and the POST1 (6.04±1.98), at FZ region ($p<0.05$). Also at this region, the alpha band increased significantly ($p<0.05$). The relative power of the alpha band (3.97±1.54) at FZ in the PRE status increased (6.05±1.43). The beta band in this region did not change significantly. At F4 region, there was no significant change in all EEG rhythms. Also there was no significant in all EEG rhythms in F4, and F8 regions.

As shown in Figure 3, there was no significant difference between the PRE in comparison to the sham status at T3, C4, and T4 regions for the beta EEG rhythms. Also there was no significant change in the beta band at T3, C3,Cz, C4 and T4. The alpha at T3, T4, andCz, between the PRE, and the POST1 statuses. An increment in the alpha relative power between the PRE, and the POST1 statuses wasincreased significant at C3, Cz, and C4($p<0.05$). The relative power of the theta at C3 in the PRE status (8.29±1.31) decreased in the POST1 status (6.09±2.11) significantly ($p<0.05$). The relative power of the alpha at C3 in the PRE status (3.40±0.31) increased in the POST1 status (6.68±1.01) significantly ($p<0.05$).At the CZ region, the theta band, in the PRE (11.47±2.11) status in comparison to the POST1 (7.02±1.34) status decreased significantly.

As the result shown in Figure 4, the relative power of the alpha at T5, P3, Pz,and p5 regions increased significantly. The relative power of the alpha at T5 in the PRE status (4.36±0.38) increased in the POST1 status (8.01±1.41) significantly ($p<0.05$). The relative power of the alpha at P3 in the PRE status (4.68±1.31) increased in the POST1 status (8.02±1.01) significantly ($p<0.05$). The other EEG rhythms in the T5, P3, PZ, and P5 regions did not change significantly.

As the results in Figure 5, the relative power of the alpha at O1 in the PRE status (5.40±0.91) increased in the POST1 status (11.61±1.03) significantly ($p<0.05$). Also, the relative power of the alpha at O2 in the PRE status (5.40±0.91) increased in the POST1 status (11.61±1.03) significantly ($p<0.05$). The other EEG rhythms in the O1, and O2 regions did not change significantly.

DISCUSSION

In this study, we attempted to examine a novel method in psychology therapy. It seems that the executed method is promising to investigating the transcranial laser stimulation effects in human brain activity. The idea is based on numerous studies oneffects of laser on mitochondria, and cells [23, 24, 18, 19]. Also, new research has confirmed the reinforcement of living cells as excitatory, in the presence of laser exposure in some frequencies [18, 19]. This study was motivated by research which showed LLL on living organisms and the related variations in the ion concentrations within the cells, when the frequency of the applied field is matched with the angular frequency. As we described before, De Ninno *et al*. [11] described that the Larmor frequency of most of the involved ions, lies between 10 and 50 Hz. Also Hamblin *et. al*. [18, 19] showed that when laser is radiated to a nerve by 0Hz, 10Hz, 100Hz, 1000Hz, and 10000Hz, the most effective frequency is 10 Hz. Also as we found out, there are so many researches that indicate the effect of 10Hz stimulation on brain activity. As demonstrated in this study, the alpha rhythm was changed significantly in many regions after exposure. As the result indicates, all the EEG rhythms at the FP1, and FP2 changed significantly ($p<0.05$). The relative power of the alpha band at FP1 at the PRE status (2.92±2.02) increased in the POST1 status (4.96±1.78) significantly ($p<0.05$). Also, the relative power of the alpha band at FP2 at the PRE status (3.20±1.02) increased in the POST1 status (5.02±0.98) significantly ($p<0.05$). The alpha band of EEG rhythm increased significantly at F7 in the PRE status (2.92±1.23) in comparison to the POST1 (4.62±0.98). The alpha band of EEG rhythm increased significantly at F3 in the PRE status (3.77±1.93) in comparison to the POST1 (5.64±1.08). Also at FZ region, the alpha band increased significantly ($p<0.05$). The relative power of the alpha band (3.97±1.54) at FZ in the PRE status increased (6.05±1.43). Even in far regions, the relative power of the alpha at O1 in the PRE status (5.40±0.91) increased in the POST1 status (11.61±1.03) significantly ($p<0.05$). Also, the relative power of the alpha at O2 in the PRE status (5.40±0.91) increased in the POST1 status (11.61±1.03) significantly ($p<0.05$).As the interaction of spin-correlated radical pairs with magnetic fields confirms that Larmor frequency coupling is due to magnetic effects, in this paper there are significant changes in the EEG rhythms as the most significant change. Some studies describes that Larmor frequency of the Fe^{2+} ion and Cu^{2+} are 17 Hz and 15 Hz, respectively. It seems that every cell or ion has a magnetic resonance, electromagnetic resonance, and electric resonance. In some studies even local extremely low frequency magnetic field [2, 31] showed significant changes in different EEG bands in other regions of the brain were observed but less than those in whole exposures. In distant regions from local exposure point, significant changes were observed as well as we did in O1, and O2 regions.

As described before, there are many studies indicating that the frequency of exposure is more important than the amplitude and time.

As can be obtained from the results, by 10Hz radiation of the brain by the super pulse infrared (904 nm) laser, in almost all parts of the brain, the alpha rhythm changed.

The result indicates the nonlinear effects may be seen and more linearity may be observed under the exposure region. The power of EEG rhythms under and near to the exposure region changed more. We know that the theta is related in internal orientation important in memory recall and other mental recalls [25, 15]. Also it is focused on external learning stimuli such as reading or listening. The alpha rhythms is internally oriented and may be observed in some types of meditation. It is possible but rare to have a dissociative experience when totally in this state. The alpha can correlate with a very alert broad awareness states. This can be a readiness state seen especially in high level outlets. Persons with high intelligence often demonstrate a higher peak Alpha frequency. The beta rhythm correlates with active problem solving cognitive activity [25, 15]. Therefore, maybe by the transcranial laser radiation we can stimulate the brain as we did by current or magnetic forces. It is extremely easy to use, without any side effect [18, 19].

LLL has shown to act by many cellular mechanisms. It increases micro and macro circulation under the exposure region. LLLT results in decreasing chemical inflammatory mediators such as prostaglandin E2, leucocytes, and tumor necrosis factor (TNFα). Photons can be delivered to distant tissues through the blood or the lymphatic system and by cell communication, the blockage of axonal flow can be altered, a decrease in pain sensation after LLLT exposure was demonstrated as well. LLL can furthermore be modulated by frequency (i.e. 10Hz). - In this study we examined the proved evidence of effect on the brain by EEG analysis. We found out that this radiation has significant effect on local region. The most affected regions were frontal regions. As result indicates all the EEG rhythms at the FP1, and FP2 changed significantly ($p<0.05$). The relative power of the theta band at FP1 at the PRE status (5.10±1.12) increased in the POST1 status (8.33±0.78) significantly ($p<0.05$). Our results may indicate that LLLTis an effective way to targeted therapeutic alteration of brain function and may become a save new tool for neuro - therapy. The use of the extremely easy method of transcranial laser stimulation in comparison to tDCS, and rTMS offers an effective user-friendly method. Randomized, controlled trials proved good evidences by LLL effect on stroke, tinnitus, pain, etc. More studies are needed to open the gates of this method into neurology, and psychiatry. This pilot study may reinforce this idea.

CONCLUSION

Transcranial LLL may be a new physical method in neuro-therapy and psychiatry, a technique that can effect on cortex and subcortical regions. The facilitation of neuron activity can increase the chemical process between neurons.

Transcranial LLL is a save method and can be applied easily and the effects measured by EEG. This study demonstrates effects on EEG rhythms such that increment in brain activity via affecting theta, alpha or beta rhythms. In this study pulsed LLL radiation was appliedon dorso lateral prefrontal cortex region (DLPFC) which is important formany human brain networks.

In comparison with tDCS or rTMS, etc., application of tLLL is very simple. Specific devices could be designed and investigated in future studies.

More studies are needed to examine transcranial persistency of LLL and long-term effects.Brain frequency response in EEG to different frequencies by LLL need to be studied in larger cohorts. The significance of the ability of transcranial low level laser to excite human cerebral cortex or subcortical effects and their correlation with clinical and behavioral treatment is yet unclear, so the methodology of this paper can be used to evaluate the specific effects. More detailed information of beta, theta, and other EEG rhythms would be published in future.

ACKNOWLEDGMENTS

We thank the reviewer for any review and highly appreciate the comments and suggestions, which significantly contributed to improving the quality of the publication. We would like to express my deep gratitude to Professor Dr. Frank Bahr, Dr. Michael Hans Weber, and Michael Hamblinfor their attempts to spread laser knowledge all over the world. We would also like to extend my thanks to the technicians of the laboratory for their help in running the study.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) received no financial support for the research, authorship, and/or publication of this article.

REFERENCES

1. Ahluwalia, G. (2008). Cosmetics Applications of Laser and Light-Based Systems, William Andrew.
2. Amirifalah, Z., S. M. P. Firoozabadi, et al. (2013). "Local Exposure of Brain Central Areas to a Pulsed ELF Magnetic Field for a Purposeful Change in EEG." Clinical EEG and neuroscience**44**(1): 44-52.
3. Avci, P., T. T. Nyame, et al. (2013). "Low-level laser therapy for fat layer reduction: A comprehensive review." Lasers in surgery and medicine**45**(6): 349-357.
4. Bassett, D. S. and E. Bullmore (2006). "Small-world brain networks." The neuroscientist**12**(6): 512-523.

5. Bjordal, J. M., R.-J. Bensadoun, et al. (2011). "A systematic review with meta-analysis of the effect of low-level laser therapy (LLLT) in cancer therapy-induced oral mucositis." Supportive Care in Cancer**19**(8): 1069-1077.
6. Bjordal, J. M., R. A. Lopes-Martins, et al. (2008). "A systematic review with procedural assessments and meta-analysis of low level laser therapy in lateral elbow tendinopathy (tennis elbow)." BMC Musculoskeletal Disorders**9**(1): 75.
7. Blanco, N. J., W. T. Maddox, et al. (2017). "Improving executive function using transcranial infrared laser stimulation." Journal of neuropsychology**11**(1): 14-25.
8. Bullmore, E. and O. Sporns (2009). "Complex brain networks: graph theoretical analysis of structural and functional systems." Nature reviews neuroscience**10**(3): 186.
9. Chow, R. T., M. I. Johnson, et al. (2009). "Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomised placebo or active-treatment controlled trials." The Lancet**374**(9705): 1897-1908.
10. Coben, R. and J. R. Evans (2010). Neurofeedback and neuromodulation techniques and applications, Academic Press.
11. De Ninno, A., M. Prosdocimi, et al. (2008). "Effect of ELF em fields on metalloprotein redox-active sites." arXiv preprint arXiv:0801.2920.
12. DeTaboada, L., S. Ilic, et al. (2006). "Transcranial application of low-energy laser irradiation improves neurological deficits in rats following acute stroke." Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery**38**(1): 70-73.
13. Edmonds, D. (1993). Larmor precession as a mechanism for the biological sensing of small magnetic fields. Electricity and magnetism in biology and medicine, San Francisco Press San Francisco**:** 1-4.
14. Enwemeka, C. S., J. C. Parker, et al. (2004). "The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study." Photomedicine and Laser Therapy**22**(4): 323-329.
15. Fernández, T., T. Harmony, et al. (2007). "Changes in EEG current sources induced by neurofeedback in learning disabled children. An exploratory study." Applied psychophysiology and biofeedback**32**(3-4): 169-183.
16. Fregni, F. and A. Pascual-Leone (2007). "Technology insight: noninvasive brain stimulation in neurology—perspectives on the therapeutic potential of rTMS and tDCS." Nature Reviews Neurology**3**(7): 383.
17. Gam, A. N., H. Thorsen, et al. (1993). "The effect of low-level laser therapy on musculoskeletal pain: a meta-analysis." Pain**52**(1): 63-66.
18. Hamblin, M. R., T. Agrawal, et al. (2016). Handbook of low-level laser therapy, CRC Press.
19. Hamblin, M. R. and Y. Huang (2013). Handbook of photomedicine, Taylor & Francis.
20. Hashmi, J. T., Y. Y. Huang, et al. (2010). "Role of low-level laser therapy in neurorehabilitation." Pm&r**2**: S292-S305.
21. Huang, Y. Y., A. Gupta, et al. (2012). "Transcranial low level laser (light) therapy for traumatic brain injury." Journal of biophotonics**5**(11-12): 827-837.
22. Ilic, S., S. Leichliter, et al. (2006). "Effects of power densities, continuous and pulse frequencies, and number of sessions of low-level laser therapy on intact rat brain." Photomedicine and Laser Therapy**24**(4): 458-466.
23. Karu, T. (1987). "Photobiological fundamentals of low-power laser therapy." IEEE Journal of Quantum Electronics**23**(10): 1703-1717.
24. Karu, T. (1988). "Molecular mechanism of the therapeutic effect of low-intensity laser radiation." Lasers Life Sci**2**(1): 53-74.
25. Klimesch, W. (1999). "EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis." Brain research reviews**29**(2-3): 169-195.
26. Konstantinović, L. M., M. B. Jelić, et al. (2013). "Transcranial application of near-infrared low-level laser can modulate cortical excitability." Lasers in surgery and medicine**45**(10): 648-653.
27. Luigjes, J., R. Breteler, et al. (2013). "Neuromodulation as an intervention for addiction: overview and future prospects." Tijdschrift voor psychiatrie**55**(11): 841-852.
28. Mehran, Y. Z., M. Firoozabadi, et al. (2013). "Analysis of EEG rhythms under local sinusoidal ELF magnetic field exposure: An approach to neurofeedback enhancement on attention performance." Journal of Biomedical Science and Engineering**6**(10): 947.
29. Moreno-Duarte, I., N. Gebodh, et al. (2014). Transcranial electrical stimulation: transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS), transcranial pulsed current stimulation (tPCS), and transcranial random noise stimulation (tRNS). The Stimulated Brain, Elsevier**:** 35-59.
30. Naeser, M. A. and M. R. Hamblin (2011). Potential for transcranial laser or LED therapy to treat stroke, traumatic brain injury, and neurodegenerative disease, Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA.
31. Shafiei, S., S. Firoozabadi, et al. (2012). "Study of the frequency parameters of EEG influenced by zone-dependent local ELF-MF exposure on the human head." Electromagnetic Biology and Medicine**31**(2): 112-121.
32. Siebner, H. R., N. Lang, et al. (2004). "Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex." Journal of Neuroscience**24**(13): 3379-3385.
33. Silveira, P. C., L. A. da Silva, et al. (2009). "Evaluation of mitochondrial respiratory chain activity in muscle healing by low-level laser therapy." Journal of Photochemistry and Photobiology B: Biology**95**(2): 89-92.

34. Silveira, P. C., E. L. Streck, et al. (2007). "Evaluation of mitochondrial respiratory chain activity in wound healing by low-level laser therapy." Journal of Photochemistry and Photobiology B: Biology**86**(3): 279-282.
35. Teggi, R., C. Bellini, et al. (2009). "Transmeatal low-level laser therapy for chronic tinnitus with cochlear dysfunction." Audiology and Neurotology**14**(2): 115-120.
36. Ueda, Y. and N. Shimizu (2003). "Effects of pulse frequency of low-level laser therapy (LLLT) on bone nodule formation in rat calvarial cells." Journal of clinical laser medicine & surgery**21**(5): 271-277.
37. Utz, K. S., V. Dimova, et al. (2010). "Electrified minds: transcranial direct current stimulation (tDCS) and galvanic vestibular stimulation (GVS) as methods of non-invasive brain stimulation in neuropsychology—a review of current data and future implications." Neuropsychologia**48**(10): 2789-2810.
38. Woodruff, L. D., J. M. Bounkeo, et al. (2004). "The efficacy of laser therapy in wound repair: a meta-analysis of the literature." Photomedicine and laser surgery**22**(3): 241-247.
39. Xuan, W., F. Vatansever, et al. (2014). "Transcranial low-level laser therapy enhances learning, memory, and neuroprogenitor cells after traumatic brain injury in mice." Journal of biomedical optics**19**(10): 108003.
40. Xuan, W., F. Vatansever, et al. (2013). "Transcranial low-level laser therapy improves neurological performance in traumatic brain injury in mice: effect of treatment repetition regimen." PloS one**8**(1): e53454.
41. Zandi Mehran, Y., M. Firoozabadi, et al. (2013). "Brain inconspicuous effect by local sinusoidal extremely low frequency magnetic exposure based on wavelet packet analysis: innovation in online passive neurofeedback therapy by the neuro-LSELF system." Journal of Neurotherapy**17**(4): 226-247.
42. Zandi Mehran, Y., M. Firoozabadi, et al. (2015). "Improvement of neurofeedback therapy for improved attention through facilitation of brain activity using local sinusoidal extremely low frequency magnetic field exposure." Clinical EEG and Neuroscience**46**(2): 100-112.