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TRANSCRANIAL INFRARED LASER STIMULATION PRODUCES BENEFICIAL COGNITIVE AND EMOTIONAL EFFECTS IN HUMANS

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Abstract—This is the first controlled study demonstrating the beneficial effects of transcranial laser stimulation on cognitive and emotional functions in humans. Photobiomodulation with red to near-infrared light is a novel intervention shown to regulate neuronal function in cell cultures, animal models, and clinical conditions. Light that intersects with the absorption spectrum of cytochrome oxidase was applied to the forehead of healthy volunteers using the laser diode CG-5000, which maximizes tissue penetration and has been used in humans for other indications. We tested whether low-level laser stimulation produces beneficial effects on frontal cortex measures of attention, memory and mood. Reaction time in a sustained-attention psychomotor vigilance task (PVT) was significantly improved in the treated ($n = 20$) vs. placebo control ($n = 20$) groups, especially in high novelty-seeking subjects. Performance in a delayed match-to-sample (DMS) memory task showed also a significant improvement in treated vs. control groups as measured by memory retrieval latency and number of correct trials. The Positive and Negative Affect Schedule (PANAS-X), which tracks self-reported positive and negative affective (emotional) states over time, was administered immediately before treatment and 2 weeks after treatment. The PANAS showed that while participants generally reported more positive affective states than negative, overall affect improved significantly in the treated group due to more sustained positive emotional states as compared to the placebo control group. These data imply that transcranial laser stimulation could be used as a non-invasive and efficacious approach to increase brain functions such as those related to cognitive and emotional dimensions. Transcranial infrared laser stimulation has also been proven to be safe and successful at improving neurological outcome in humans in controlled clinical trials of stroke. This innovative approach could lead to the development of non-invasive, performance-enhancing interventions in healthy humans and in those in need of neuropsychological rehabilitation.

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Abbreviations: ANOVA, analysis of variance; DMS, delayed match-to-sample; LEDs, light-emitting diodes; LLLT, low-level light therapy; OD, optical density; PANAS, Positive and Negative Affect Schedule; PEBL, psychology experiment building language; PVT, psychomotor vigilance task; SSS, sensation-seeking scale; TPQ, Tri-Dimensional Personality Questionnaire.

Key words: transcranial laser stimulation, low-level light therapy, attention, memory, mood, novelty-seeking.

INTRODUCTION

The goal of this experiment was to use transcranial low-level light therapy (LLLT) to enhance frontal cortex cognitive and emotional functions. LLLT is defined as the use of directional low-power and high-fluence monochromatic or quasimonochromatic light from lasers or light-emitting diodes (LEDs) in the red to near-infrared wavelengths to modulate a biological function or induce a therapeutic effect (Rojas and Gonzalez-Lima, 2011). LLLT is non-invasive, therapeutically beneficial, and promotes a wide range of biological effects including the enhancement of energy production, gene expression and the prevention of cell death. Previous research has indicated that depressed patients showed a beneficial effect on their affective state from a single LLLT treatment to the forehead using 810 nm LEDs (Schiffer et al., 2009). The present experiment tested whether LLLT benefits extend to cognitive processes involving attention, vigilance and short-term memory, and if there may be a relationship between response to LLLT and personality measures. Instead of using LEDs, we administered LLLT with a 1064-nm laser that maximizes tissue penetration (Sommer et al., 2001).

Stimulation with red to near-infrared light constitutes a novel intervention shown to regulate neuronal function in cell cultures, animal models, and clinical conditions (Eells et al., 2004). Photobiomodulation of mitochondrial cytochrome oxidase activity appears to be the primary molecular mechanism of action of LLLT. Cytochrome oxidase is the primary photoacceptor of red to near-infrared light energy, and it is also the enzyme catalyzing oxygen consumption in cellular respiration (Karu, 2000; Wong-Riley et al., 2005) and the production of nitric oxide under hypoxic conditions (Poyton et al., 2009). We have previously shown that transcranial LLLT can increase cytochrome oxidase activity in the rat brain (Rojas et al., 2008), which can provide neuroprotection against toxicity in animal models (Rojas and Gonzalez-Lima, 2010, 2011). LLLT *in vivo* can also increase cytochrome oxidase and improve the aerobic capacity of other tissues such as skeletal muscle (Hayworth et al., 2010). We recently demonstrated that transcranial LLLT can improve frontal cortex oxygen consumption and metabolic capacity and

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thereby increase frontal cortex-based memory functions in rats (Rojas et al., 2012). These findings in animals suggest that the oxidative metabolism of tissue exposed to LLLT is enhanced. LLLT also appears to have *in vivo* metabolic effects in human brain and muscle tissues. For example, LLLT has been used non-invasively in humans to stimulate the brain as an antidepressant treatment (Schiffer et al., 2009) and improve neurological outcome after ischemic stroke (Lampl et al., 2007), as well as to alleviate muscle fatigue and enhance recovery (Leal Junior et al., 2010). These LLLT treatments have thus been proven to be not just safe but actually beneficial in humans. In particular, Schiffer et al. (2009) found that a single LLLT treatment to the forehead resulted in a significant beneficial effect in patients with major depression and anxiety. No adverse side effects were found in any of the patients, either immediately after the initial treatment, or at 2 or 4 weeks post-treatment. We followed a similar transcranial LLLT protocol to the forehead, targeting frontal cortex-based cognitive tasks such as a psychomotor vigilance task (PVT) and a delayed match-to-sample memory task (DMS) immediately after LLLT, and also assessed emotional states 2 weeks after LLLT.

The PVT (Dinges and Powell, 1985) is a test that assesses an individual's sustained attention. The PVT involves the subject maintaining a vigilant state during a delay period, then responding as fast as possible when a stimulus appears onscreen. These attentional processes are mediated by the frontal cortical regions (Marklund et al., 2007) targeted by the LLLT treatments in this experiment, and the PVT has been shown to be a reliable indicator of frontal function (Drummond et al., 2005). Another test, the DMS task, has been shown to be mediated by a frontoparietal network (Nieder and Miller, 2004). This task involves the presentation of a visual stimulus on a screen. Then the stimulus disappears, and the participant must remember the stimulus through a delay. Then two choices appear, and the participant must decide which of these two is identical to the previous stimulus (the "match"). Prefrontal cortical neurons are specifically active during the delay portion of the DMS task (Sawaguchi and Yamane, 1999). It is possible that by augmenting the metabolism of these frontal cortex regions, efficiency on the PVT and DMS tasks could increase as well.

Questionnaires were used to evaluate aspects of mood and personality. The self-reported emotional states of participants, before and after LLLT, were measured using a version of the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988), specifically, the PANAS-X (Watson and Clark, 1999), which tracks positive and negative emotional states over time. Participants filled out the PANAS for the first time immediately prior to LLLT, and again at 2 weeks post-treatment, to determine if there was a long-lasting beneficial effect of LLLT on mood. The Tri-dimensional Personality Questionnaire (TPQ; Cloninger, 1987), which evaluates dimensions such as novelty-seeking, reward dependence, and harm avoidance, and the Sensation-Seeking Scale, form V (SSS; Zuckerman, 1994), which measures sensation-seeking tendencies, were used to see if there was a predictive relationship between treatment response to LLLT and aspects of personality.

The two independent variables were sex and group (treated vs. control). The dependent variables were response times in the PVT; correct vs. failed trials in the PVT; memory retrieval latencies in the DMS task; and correct vs. failed trials in the DMS task. Having a pre-test (immediately before LLLT treatment) and a post-test (immediately after LLLT treatment) for both of these tasks allowed us to control for individual differences in familiarity/skill with the tasks. Another dependent variable (the scores on the PANAS, pre-test vs. post-test) similarly allowed for a pre-treatment vs. post-treatment comparison, to look for any long-term effects of LLLT on mood as seen in Schiffer et al. (2009). Because Schiffer et al. (2009) found a beneficial effect specifically in depressed patients, subjects were also given a brief medical history questionnaire, to determine if they had a history of depression or anti-depressant usage. However, subjects were not recruited on this basis, and non-depressed subjects were not excluded from the analysis.

While the primary purpose of this study was to evaluate whether LLLT had an effect on frontal cortex measures of attention, memory and mood in humans, a series of follow-up measures using a post-mortem human skull from the university collection were also conducted to provide an estimate of the percentage of light that travels to the frontal cortical surface. Different wavelengths of interest may be transmitted transcranially at different rates (Nickell et al., 2000), and unfortunately, experimental measurements of the optical properties of biological tissue often find little agreement between different observers (Bernini et al., 1991). By independently measuring the intensity of the laser beam before and after it had passed through this tissue, as well as the width of the tissue itself, Beer's Law could be used to calculate the approximate optical density and absorption coefficient. These follow-up experiments were descriptive only and not intended to test any hypothesis, but rather to provide estimates of the penetration of the 1064-nm wavelength laser.

EXPERIMENTAL PROCEDURES

Human subjects

The protocol was approved by the University of Texas at Austin's Institutional Review Board and complied with all applicable federal and NIH guidelines. Healthy, English-speaking adults of either sex, of age ranging from 18 to 35 years, of any ethnic background were considered for the study. Potential subjects were recruited using a posting in the online subject pool management system known as OPERA, an online tool in which undergraduates currently enrolled in an introductory psychology class at the University of Texas at Austin participate in experiments in exchange for course credit. The exclusion criteria for subject participation were as follows: diagnosis of psychotic disorder, history of violent behavior, history of neurological condition, current pregnancy, or prior institutionalization or imprisonment; however, no participant

was excluded on these bases. Participants were recruited over the course of a semester until the target number of subjects per group was reached (n = 10 male, treated; n = 10 male, control; n = 10 female, treated; n = 10 female, control). Treatment group was randomly assigned prior to human subject interaction.

Procedure for obtaining informed consent

The experimenter obtained informed consent from participants at the beginning of the experimental session. The explanation/consent form included details about the safety procedures used in the operation of the CG-5000 laser used to conduct the LLLT. Participants were told directly (and in the consent form) the rationale for the experiment, to measure the effects of LLLT on sustained attention and mood. Participants were told that they might be a part of either the experimental (treated) or control (placebo) groups, that they would not be told which, but that they might inquire as to which group they were assigned after their participation in the experiment had concluded. After this explanation, participants were given the chance to opt out of the experiment with no repercussions, but none did.

Pre-treatment experimental protocol

To prevent distraction during the tests, participants were asked to surrender their backpack and any electronic devices. Participants supplied their name, age, sex, race, handedness, and email address, and were assigned a random 4-digit number. After signing the consent form, participants were led to a quiet, closed room, and given the short medical history form, the PANAS (pre-treatment), TPQ, and SSS questionnaires to fill out.

The Positive and Negative Affect Schedule, or PANAS-X (Watson and Clark, 1999), which tracks self-reported positive and negative affective (emotional) states over time, was administered immediately before treatment and 2 weeks after treatment. Participants read a series of adjectives which describe an emotional state, then scored each on a scale of 1–5 on how frequently they experienced that emotional state within the previous 2 weeks. The cumulative ''positive'' affect score was the sum of the scores given to each of the following adjectives: active, alert, attentive, determined, enthusiastic, excited, inspired, interested, proud, and strong. The cumulative ''negative'' affect score was the sum of the scores given to each of the following adjectives: afraid, scared, nervous, jittery, irritable, hostile, guilty, ashamed, upset, and distressed. More details on scoring of the PANAS-X can be found in Watson and Clark (1999) and in Crawford and Henry (2004). The Tri-dimensional Personality Questionnaire (TPQ; Cloninger, 1987), which consists of 100 forced-choice (true vs. false) questions, evaluates dimensions such as novelty-seeking, reward dependence, and harm avoidance. The SSS, form V (SSS; Zuckerman, 1994), which consists of 40 forced-choice (A vs. B) questions, measures sensation-seeking tendencies. TPQ and SSS were used to see if there was a predictive relationship between treatment response to LLLT and aspects of personality. These questionnaires were later converted into numerical scores by an experimenter unaware of participant identity or group assignment; the medical history was scored as either 1 (a history of depression, anti-depressant use, or suicide attempt) or 0 (no such history).

The PVT and DMS tasks were implemented by the Psychology Experiment Building Language (PEBL), an open-source programming language. One desktop computer in the lab was designated as the testing apparatus. Participants were identified by their randomly-assigned subject number typed into the program, prior to the start of each block of trials. The data gathered during the PVT included each trial's intertrial interval in seconds, reaction time in milliseconds, and a code number indicating whether the trial was a success (response in less than 30 s), a lapse (no response in 30 s), or a false alarm (response with a button press prior to the onset of the cue).

Immediately following the questionnaires, subjects were given a short (1-min) practice session of the PVT, to familiarize them with the task. They then participated in one block of the PVT. The PVT (Dinges and Powell, 1985) is a test in which participants attend to a small fixation point which appears briefly at the center of a computer screen, then disappears. Then, at random intervals, a bright millisecond timer appears in the center of the screen. Participants were instructed to respond via button press as rapidly as possible upon detection of the counter stimulus; the participant's response stopped the counter from updating. The final counter value corresponded to the participant's reaction time and was displayed onscreen for 1 s, thus providing feedback for that particular trial. Participants were given 30 s to make a response before the computer aborted a trial, though no participant showed any evidence of such a lapse. Information about each trial's success/failure and reaction time was stored by the computer for later analysis, and indexed by the subject's randomly-assigned number. The block of PVT trials consisted of 40 trials (approximately five minutes long); intertrial intervals were randomly chosen without replacement from between 2 and 10 s; thus, the average intertrial interval was around 6 s. The post-treatment block of PVT trials was identical to the first.

The participant then took part in the DMS task, which also measures reaction time, but has a short-term memory component as well. As with the PVT, participants were first given a short (1-min) practice session of the DMS, to familiarize them with the task. This task entailed viewing a 4×4 grid of brightly colored squares with a unique, randomly-generated pattern for each of 30 trials (approximately five minutes). The grid of 16 squares consisted of 7, 8, or 9 red-colored squares, with yellow squares comprising the rest of the grid. Then, with a key press, that stimulus disappeared, and the screen was blank through a delay (4 s). Two stimuli were then presented on screen (a ''match'' and ''nonmatch''), on the left and right of the screen. The ''match'' was identical to the previous stimulus, while the ''non-match'' contained 1–2 randomly switched squares. The participant indicated which stimulus was the correct ''match'' with a key press. ''Correct'' or ''Incorrect'' was displayed for one second after each trial to provide feedback. Correct/incorrect status and memory retrieval latency (reaction time) for each trial were measured by the computer and stored for later analysis. The post-treatment block of 30 DMS trials was identical to the first. The participants were informed that while there was no time limit for either studying the target or choosing the match, they should attempt to ''be as fast as possible, while still being accurate.''

Laser treatment and post-treatment experimental protocol

After the block of DMS testing, the LLLT was administered. This treatment consisted of applying light of a specific wavelength (1064 nm) that intersects with the absorption spectrum of cytochrome oxidase, using a laser diode supplied by Cell Gen Therapeutics, LLC (Model CG-5000 laser, HD Laser Center, Dallas, TX, USA). This device has not been evaluated or approved by the FDA for the specific uses tested in this study. Marketing of the Cell Gen Model CG-4000 laser in the USA is FDA-cleared as safe for various uses on humans, such as for improving circulation, temporary relief of muscle and joint pain, muscle spasm, stiffness associated with arthritis, and relaxation of muscle tissue. The laser received approval from the University of Texas at Austin Laser Safety Program and a standard operating procedure for the laser was approved by the University Laser Safety Officer. Both participants and

experimenters wore protective eyewear, though the administrators of the LLLT were careful not to shine the light in the eyes.

The irradiance (or power density) used, 250 mW/cm^2, as well as the cumulative fluence (or energy density) used, 60 J/cm^2, are the same parameters that showed psychologically beneficial effects in Schiffer et al. (2009). The laser treatment was continuous, not pulsed. At the power level described, the energy emitted by the CG-5000 is low, exposure to it is not harmful to tissue, and it causes negligible heat and no physical damage. Similar settings are used clinically by Cell Gen Therapeutics for the treatment of lower back pain, sciatica, and migraine headaches.

The LLLT treatment occurred in a locked room with black walls and no reflective surfaces. The experimenter locked both himself and the participant inside the room, put a sign on the outer door indicating that the apparatus was in use, and made sure that protective eyewear (900–1000 nm: 5+, 1000–2400 nm: 7+; 2900–10600 nm: 7+) was worn by both individuals. The laser's power output is automatically calibrated by an internal mechanism; however, in addition to this calibration, the power density in mW/cm^2 (and thus the energy density dose in J/cm^2) was confirmed independently using a Newport model 1916-C power meter attached to a Newport model 918D-SL photodiode detector, prior to the experimental sessions.

The laser was directed at the right frontal pole of the cerebral cortex, which is the most anterior region of the right prefrontal cortex (Brodmann's areas 9 and 10). In reference to the 10–20 system used for EEG electrode placement, the forehead stimulation site in our experiment was centered on the FP2 (right frontal pole) point. The laser stimulation extended medially for a 4-cm diameter area from this point, and laterally for another 4-cm diameter area from this point. The location of the stimulation on the forehead was like that shown in Fig. 1 of Schiffer et al. 2009, the first paper which showed a beneficial effect of near-infrared light on mood; however, our experiment targeted the right side of the forehead only, since the right frontal pole region is implicated in sustained attention (Sturm and Willmes, 2001; Lawrence et al., 2003; Drummond et al., 2005).

In addition to the protective eyewear provided, subjects were instructed to keep their eyes closed. The CG-5000 has a handheld 4-cm diameter aperture that can be aimed by the experimenter, with a button on the handle that controls the onset and offset of the photodiode. Each one-minute treatment cycle was marked by a timer counting down and by a beep from the apparatus. Each participant received four one-minute treatments to each of two sites on the right forehead, alternating between sites medial and lateral to the FP2 point. Thus the entire treatment lasted for 8 min in total. The vascularity of the scalp efficiently removed heat and prevented any significant heat accumulation.

The control group underwent the same procedure as the treatment group, but received a brief (5-s) treatment to the intended site on the forehead, followed by 55 s of no treatment, for each one-minute cycle. Thus the control group received approximately 1/12th of the cumulative energy density as the treatment group. This 5-s treatment was sufficient to provide a brief sensation of slight heat (as active placebo) at the onset of each one-minute cycle, using a fraction of the energy received by the experimental group.

After the LLLT treatment, the participants again took part in another 5-min block of the PVT and another 5-min block of the DMS, identical to the first two blocks. These post-treatment tests were compared to the pre-treatment tests to determine any effects of the LLLT. The duration of the entire session was between 1–1½ hour, depending on how long it took the participants to complete the questionnaires.

Two weeks later, participants were contacted by email and sent a copy of the PANAS, to be filled out a second time. The Institutional Review Board of the University of Texas at Austin approved the use of e-mail communication as a valid method for this population for this purpose. Subjects were instructed to evaluate their emotional states for the intervening two-week period, i.e., the period of time after the LLLT session. They were also asked if they had experienced any perceived physical, mental, or health-related side effects of the LLLT treatment during this time. The responses were used to determine whether any long-lasting psychological benefit had been conferred from the LLLT, and ensure that there have been no detrimental effects of the LLLT. One participant, who never returned the second PANAS and only performed at chance (50% correct) in the DMS task, was dropped from the study, and one additional subject was run to bring the total number of subjects to 40. The sequence of events can be found in Table 1.

Statistical analysis

First, it was determined whether each dependent variable was normally distributed, by assessing its skewness and kurtosis. Normally-distributed variables were analyzed with repeated measures ANOVA (analysis of variance), using pre-post treatment measures as the within-subject variable, and group assignment (treated vs. control) and sex (male vs. female) as independent variables. A significant effect of LLLT would be indicated by an interaction between the treatment group and the within-subject variable of pre-post treatment. One

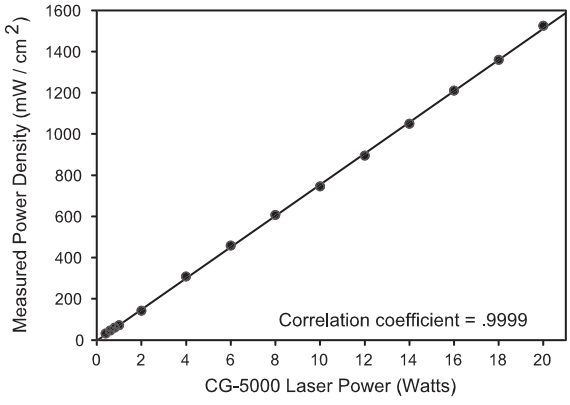

Fig. 1. Calibration curve for CG-5000 laser. Laser power output level was confirmed independently using a photodiode detector, prior to each human subject interaction.

Table 1. Experimental protocol

1	Verification of screening criteria
2	Subject information collected
3	Signing of informed consent form
4	PANAS (pre-test)
5	TPQ questionnaire
6	SSS questionnaire
7	Medical history questionnaire
8	One-minute practice of PVT
9	Block 1 of PVT (pre-test)
10	One-minute practice of DMS
11	Block 1 of DMS (pre-test)
12	LLLT
13	Block 2 of PVT (post-test)
14	Block 2 of DMS (post-test)
15	[Two weeks later] PANAS (post-test)

dependent variable, the number of false alarms on the PVT, was found to have a non-normal distribution for both pre- and post-treatment blocks of PVT (maximum skewness = 2.821, kurtosis = 10.092), likely because over half of participants had zero false alarms for both pre-treatment and post-treatment blocks of PVT. A non-parametric Mann–Whitney U-test performed on this variable found no significant effects.

RESULTS

Calibration curve

The power levels emitted by the CG-5000 were confirmed using a Newport model 1916-C power meter attached to a Newport model 918D-SL photodiode detector. (The power output is also automatically measured and calibrated by an internal mechanism, every time the treatment parameters are set by the user; the separate detector was used to confirm this calibration.) A range of power levels from 0.4 to 20 W was programmed into the laser, and the power density in mW/cm^2 was measured. Fig. 1 shows the highly linear calibration curve. The correlation coefficient was calculated as 0.9999, which was significant at $p < 0.001$, and the values were consistent with the cross-sectional areas of the beam itself (4 cm) and the aperture of the detector (1 cm).

Positive and Negative Affect Schedule (PANAS)

The PANAS showed that while participants generally reported more positive affective states than negative, overall affect improved significantly in the treated group due to more sustained positive emotional states as compared to the placebo control group. The PANAS data used a difference score (positive affect score minus negative affect score). This score was calculated for each subject's pre-treatment and post-treatment PANAS questionnaire, as a measure of overall affective valence. A repeated measures ANOVA was run using this score as the within-subject variable, with group and sex as independent variables. The resulting two-way interaction between treatment group and pre-post measures of overall affect was significant [$F(1,36) = 4.394$, $p = 0.043$], indicating that overall affect improved significantly more in the treated group, as seen in Fig. 2. The results from the PANAS show that while untreated participants generally reported a decline in positive affect over the 2 weeks following the experiment, the treated group maintained the same degree of positive affect that they initially reported.

There were no main effects of group assignment on either negative or positive affect, indicating that the random assignment of participants to one treatment group or the other was successful in balancing the groups with respect to their initial (pre-treatment) emotional states. There were no main effects of sex on either positive [$F(1,36) = 2.023$, $p = 0.164$] or negative [$F(1,36) = 0.282$, $p = 0.599$] affect, nor were there significant interactions with sex, group assignment, and pre-post measures of either positive [$F(1,36) = 2.501$, $p = 0.123$] or negative [$F(1,36) = 0.045$, $p = 0.834$] affect, indicating that males and females were not differentially affected by the treatment in terms of emotional states. As such, the effects of treatment collapsed across sex are shown in Fig. 2.

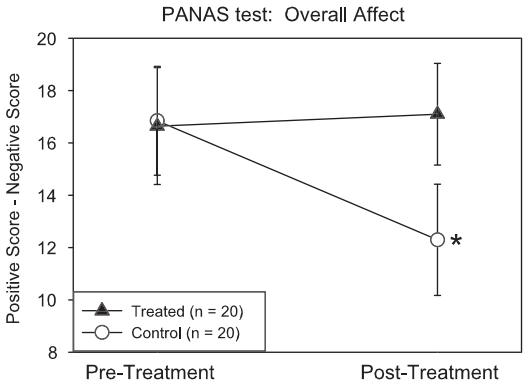

Fig. 2. Overall affect scores (calculated as positive affect score minus negative affect score) on the PANAS in the LLLT treated vs. control groups immediately before treatment and two weeks after treatment. The treated group was composed of $n = 10$ males and $n = 10$ females; the control group was composed of $n = 10$ males and $n = 10$ females. *Significant treatment by pre-post score interaction, $p < 0.05$.

Psychomotor vigilance task (PVT)

The treated group showed significant beneficial effects on the sustained attention task. The results showed that treatment improved reaction time in a sustained vigilance test, as indicated by a significant interaction between treatment and the pre-post change in reaction time [$F(1,36) = 4.211$, $p = 0.047$]. As with the PANAS, there were no main effects of group assignment on reaction time, indicating that the random assignment of participants to one treatment group or the other was successful in balancing the groups with respect to their initial (pre-treatment) times. There was a non-significant trend for a main effect of sex on reaction time in the PVT [$F(1,36) = 2.850$, $p = 0.100$], such that males tended to show faster reaction times (an average of 12 ms faster) than females, both before and after treatment.

There was no interaction of sex, group assignment, and pre-post reaction times [$F(1,36) = 1.128$, $p = 0.295$], indicating that males and females were not differentially affected in terms of reaction time on the PVT. As such, the effects of treatment collapsed across sex are shown in Fig. 3.

Trials on the PVT were considered "correct" if the participant did not have a "false alarm" (respond with a key press prior to the onset of the target) or have a "lapse" (reaction time longer than 500 s) (Dinges and Powell, 1985). The average number of correct trials was over 38 out of 40 for both blocks for all groups. The only significant finding from the analysis of the number of correct trials was a significant main effect of the within-subject change [$F(1,36) = 6.658$; $p = 0.014$], indicating that most participants improved in terms of number of correct trials from block 1 to block 2.

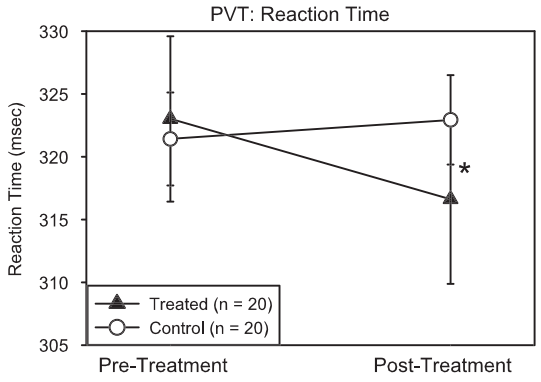

Fig. 3. Reaction time in the psychomotor vigilance task (PVT) in the LLLT treated vs. control groups measured immediately before and immediately after treatment. The treated group was composed of $n = 10$ males and $n = 10$ females; the control group was composed of $n = 10$ males and $n = 10$ females. *Significant treatment by pre-post score interaction, $p < 0.05$.

Delayed match-to-sample task (DMS)

Memory retrieval latency and correct match-to-sample trials improved significantly in the treated group. Tests for normal distribution on the pre-test and post-test memory retrieval latencies on the DMS found that these variables were not normally distributed, with kurtosis values of 1.669 (block 1) and 4.901 (block 2). This was found to be due to a single subject, identified as an outlier who was more than three standard deviations higher in both blocks of the DMS. This outlier's average memory retrieval latencies were over 3 s, which was over twice the average time for other subjects. When this outlier was removed, the skewness and kurtosis statistics remained lower than 1, and no further outliers were identified. A repeated-measures ANOVA (without the outlier) found an interaction between treatment group assignment and the within-subject variable of pre-post treatment [$F(1,35) = 5.828$, $p = 0.021$], indicating a significant effect of treatment on memory retrieval latency in the DMS (Fig. 4). There was also a main effect of the within-subject variable [$F(1,35) = 23.668$, $p < 0.001$], indicating that participants generally showed faster response times in the DMS between blocks 1 and 2. However, the significant interaction indicates that the improvement was significantly greater for the LLLT-treated group. There was no main effect of sex [$F(1,35) = 1.296$, $p = 0.263$], or interaction between sex, group, and pre-post latency [$F(1,35) = 0.180$, $p = 0.674$].

In terms of numbers of correct responses, a repeated-measures ANOVA found an interaction between treatment group assignment and the within-subject variable of pre-post treatment [$F(1,36) = 5.513$, $p = 0.012$], indicating a significant effect of treatment on the number of correct responses in the DMS (Fig. 4). There were no main effects of sex [$F(1,36) = 1.373$, $p = 0.249$], group assignment [$F(1,36) = 0.803$, $p = 0.376$], or the within-subject variable [$F(1,36) = 1.286$, $p = 0.264$]; the lack of a main effect of the pre-post within-subject variable indicates that participants did not necessarily benefit from practice on the DMS. In fact, the significant interaction was driven more by a decrease in correct trials for the control group, perhaps as the result of fatigue on this task.

Pigmentation

To determine if skin pigmentation level made a difference on treatment effects, subjects were classified as having either dark pigmentation (subjects with brown to black skin; $n = 13$) or light pigmentation (subjects with white skin; $n = 27$), and this independent variable, along with treatment group, was included in the repeated measures ANOVAs described previously. Because treatment group was randomly assigned prior to human subject interaction, and participants were not recruited on the basis of skin pigmentation, subject numbers per group were unequal (dark: 5 treated, 8 untreated; light: 15 treated, 12 untreated) for this independent variable. A differential effect of LLLT on the basis of pigmentation would be reflected by a significant three-way interaction between treatment group, pigmentation, and the within-subject variable of pre-post treatment; however, this interaction was not significant for the results of the PANAS, PVT, or DMS (all $p > 0.7$), indicating that skin pigmentation did not appear to play a significant role in treatment response to the LLLT. Future work including a larger sample size and a more detailed means of

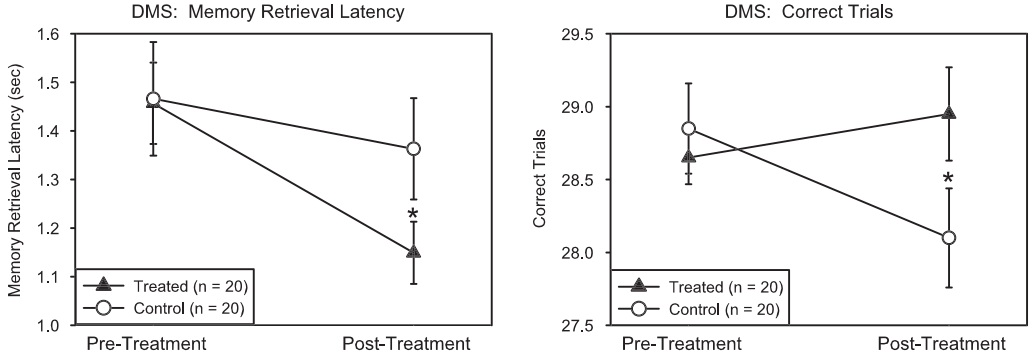

Fig. 4. Performance in the delayed match-to-sample (DMS) task in the LLLT treated vs. control groups as measured by memory retrieval latency (left panel) and number of correct trials (right panel) out of a possible 30. The treated group was composed of $n = 10$ males and $n = 10$ females; the control group was composed of $n = 10$ males and $n = 10$ females. *Significant treatment by pre-post score interaction, $p < 0.05$.

quantification of pigmentation level could address this question further.

Human transcranial transmittance

A post-mortem human skull specimen was used to provide an estimate of laser transmittance through the frontal bone. Incident light (I_o) was measured without the tissue positioned above the detector; transmitted light (I) was measured with the tissue directly overlying the aperture of the detector; the average of four readings was used to calculate percent transmittance (λ) as $100 \times I/I_o$. OD was calculated as – log (λ). The cross-sectional width of each set of tissues was measured with calipers, and Beer's Law was used to calculate the absorption coefficient (α) = OD/width. Four readings were taken from both left and right sides, then averaged together, and Beer's Law was used to calculate the absorption coefficient. Approximately 2% of the 1064-nm wavelength passed through the supraorbital frontal bone (the forehead site of the LLLT), yielding an OD of 1.70 and an absorption coefficient of $\alpha = 0.24$. This absorption coefficient is consistent with previously reported values of transmittance of this wavelength through cranial bone of $\alpha = 0.22$ (Bashkatov et al., 2006; Genina et al., 2008).

Questionnaires

Only four subjects (out of 40) reported a history of depression on the medical history questionnaire. While this finding is consistent with previously-reported rates of depression in the population at large (Lambert, 2006), there were not enough depressed subjects to perform a meaningful analysis of a possible interaction between depression history and treatment group. Future work recruiting subjects on the basis of depression susceptibility could address this question further.

As part of the follow-up questionnaire, subjects were asked if they thought they were part of the treated or control group. Though the question was phrased as forced-choice, several subjects answered with "I don't know." Of the 20 subjects in the treated group, 9 correctly believed they were treated, 8 incorrectly believed they were control group subjects, and 3 responded with "I don't know." Of the 20 subjects in the control group, 8 correctly believed they were in the control group, 7 incorrectly believed they were treated, and 5 responded with "I don't know." These roughly-equal responses indicate that these findings are unlikely to be due to the placebo effect. To verify this, the repeated-measures analyses described above were re-run, with the subjects' opinions serving as the independent variable instead of group assignment, and subjects' opinions were not found to be significant. This also ruled out that the experimenter may have unconsciously conveyed knowledge about group assignment to the participants.

To determine whether personality traits might be predictive of treatment response, all subjects were ranked according to the sensation-seeking, novelty-seeking, reward dependence, and harm avoidance scales as measured by the SSS and TPQ questionnaires. Half of the subjects were classified as high ($n = 20$) and half as low ($n = 20$) in each of the four dimensions based on a median split. The repeated-measures ANOVAs were re-run with group assignment and the four traits (high-low) as independent variables. A three-way interaction between personality dimension, treatment group, and the within-subject pre-post variable would indicate that the trait made a difference in treatment response. One analysis, using the novelty-seeking trait, and reaction time in the PVT as the dependent variable, revealed just such a three-way interaction [$F(1,36) = 4.398$, $p = 0.043$]. The three-way interaction, between novelty-seeking (high-low), treatment group, and the within-subject pre-post variable, is illustrated in Fig. 5. Reaction time in the PVT was the only dependent variable to show this three-way interaction. The low novelty-seekers showed no difference between treatment groups in the post-treatment PVT; all of these subjects were more or less unchanged. The high novelty-seekers, on the other hand, diverged on the basis of treatment. Those high novelty-seekers that were treated showed improvement on the PVT (lower reaction times), while those that were in the control group showed worse performance on the PVT (higher reaction times).

DISCUSSION

Effects of LLLT on brain and behavior

This is the first controlled study demonstrating the beneficial effects of transcranial laser stimulation on cognitive and emotional functions in healthy human volunteers. LLLT either improved, or protected against deterioration, in a number of behaviors and self-report measures linked to the functioning of the frontal cortex, including reaction time in a psychomotor vigilance task, memory retrieval latency and correct match-to-sample trials and positive emotional states. LLLT exposes a target tissue to a low-power, high-fluency source of monochromatic photon radiation, delivering energy doses that are too low to cause damage, yet high enough to modulate neuronal functions (Sommer et al., 2001; Wong-Riley et al., 2005; Rojas and Gonzalez-Lima, 2011). However, a largely unexplored research area involves LLLT effects on cognitive and emotional functions in controlled human studies. LLLT can improve working memory in middle-aged mice tested in a spatial navigation task (Michalikova et al., 2008), and there is a report of improved attention, executive function, and memory in two patients with chronic traumatic brain injury with the daily use of LLLT to the head (Naeser et al., 2010). One report in rats (Wu et al., 2012) and another in humans (Schiffer et al., 2009) provide further evidence that LLLT modulates mood and may alleviate depression. In animal models, LLLT facilitates cytochrome oxidase activity, cortical oxygenation and cerebral blood flow and thereby improves memory retention (Rojas et al., 2012) and behavioral recovery after experimental stroke (Uozumi et al., 2010). Transcranial LLLT has also been successful at improving neurological outcome in humans in controlled clinical trials of stroke (Lampl et al., 2007; Zivin et al., 2009;

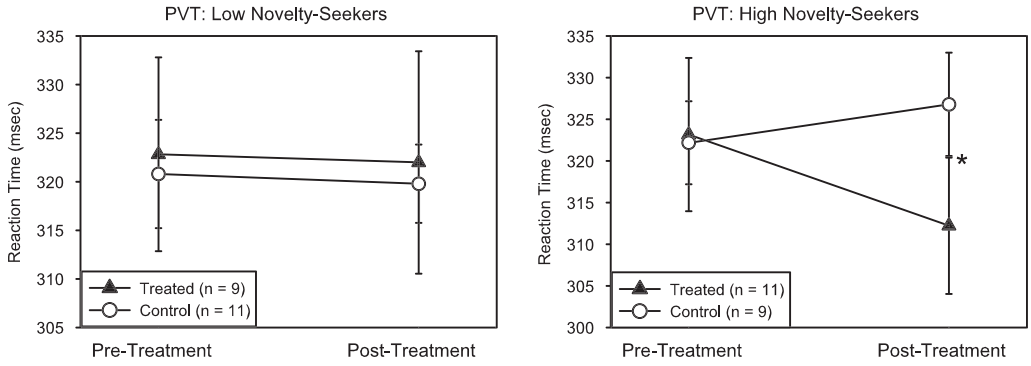

Fig. 5. Performance in the PVT task in the LLLT treated vs. control groups for subjects categorized as low (left panel) and high (right panel) novelty-seekers by their responses in the TPQ. Low novelty-seekers showed no difference in reaction time from treatment. High novelty-seekers in the control group perform worse in the post-test, while those in the treatment group perform better. *Significant treatment by pre-post score by novelty-seeking score interaction, $^{*}p < 0.05$.

Stemer et al., 2010). If proven effective, LLLT treatments could be cost-effective, safe, and non-invasive (Naeser and Hamblin, 2011).

The neuromodulatory use of red to near-infrared light wavelengths is based on the principle that certain molecules in living systems absorb photons and trigger signaling pathways in response to light. In biologic tissues, absorption and scattering of light (which would render it ineffective as a treatment) are maximal at wavelengths below 600 nm, and water absorbs light at wavelengths greater than 1150 nm. Thus, there is a ''wavelength window'' for biologic stimulation that covers the red to near-infrared light spectrum (between 600 and 1150 nm) (Hamblin and Demidova, 2006). Molecules that absorb these wavelengths in cells were discovered over 20 years ago to be components of the respiratory chain (Karu, 1989). In particular, the absorption spectra of the terminal respiratory enzyme cytochrome oxidase in different oxidation states have been found to parallel the action spectra (photoresponse as a function of wavelength) of LLLT (Karu, 2000). Thus, cytochrome oxidase is regarded as the primary cell photoacceptor of light in the red to near-infrared region of the visible spectrum (Pastore et al., 2000). In neural tissue, cytochrome oxidase is the most abundant metalloprotein, and wavelengths in its absorption spectrum correlate well with its catalytic activity and with ATP content *in vitro* (Eells et al., 2004). High bioavailability of LLLT to brain tissue *in vivo* is supported by preclinical evidence of transcranially-induced increases in brain cytochrome oxidase activity and improved behavioral outcome in normal rats and rats with impaired mitochondrial function (Rojas et al., 2008; Rojas et al., 2012). Cytochrome oxidase activity is used as a sensitive marker of brain metabolic capacity linked to neuronal activity (Wong-Riley, 1989) and behavioral outcome (Gonzalez-Lima and Cada, 1998). The neuroprotective mechanism of action of LLLT has been shown to involve direct stimulation of the catalytic activity of cytochrome oxidase and upregulation of genes involved in homeostasis, including those directly related to mitochondrial energy metabolism and intrinsic antioxidant defenses (Shefer et al., 2002; Liang et al., 2006). We previously characterized the neuroprotective effects of LLLT in an animal model of optic neuropathy. Similar to the effects of methylene blue, LLLT prevented the loss of retinal nerve fibers induced by the neurotoxin rotenone and prevented the disruption of visually guided behavior and the metabolic signs of visual deafferentiation (Rojas et al., 2008). This is evidence that highly efficient neuroprotection against mitochondrial inhibition is feasible using this non-invasive and non-pharmacologic strategy. Notably, the neuroprotective effects of LLLT were observed in a dose-dependent manner in structural and functional dimensions, and were accompanied by cerebral increases in cytochrome oxidase and superoxide dismutase activities. The last finding supports a potential application of LLLT for the non-invasive treatment not only of ophthalmologic but also intracranial neurologic conditions in humans. For example, transcranial infrared laser therapy was shown to be both safe and effective in treating human subjects that had suffered from ischemic stroke (Lampl et al., 2007; Zivin et al., 2009).

The improvement in mood after LLLT is consistent with the findings of Schiffer et al. (2009), but interestingly, the effect of LLLT on mood was mainly manifested as a protective effect against a general trend of increasing negative and decreasing positive affect over time. The participants, college students enrolled at the University of Texas at Austin, were measured during the semester, such that the post-test always occurred 2 weeks later in the semester than the pre-test. (No participant was evaluated before the beginning or after the completion of a semester.) Based on the questionnaire responses it appears that emotional stress might have generally increased over the course of the semester, with concomitant increases in negative affect / decreases in positive affect. The treatment effect seemed to work against this tendency, resulting in a protective effect whereby treated subjects showed stable levels of positive affect, while their control-group counterparts showed the tendency toward decreased positive feelings over the course of the semester. A similar protective phenomenon may have been at work on the effect of LLLT on correct responses in the DMS; the treatment

seems to have resulted not in enhanced performance in terms of correct responses, but rather protection against the effects of fatigue during block 2 of the DMS, which was the last task in the experimental session and perhaps the most likely to suffer from the effects of fatigue.

Role of the frontal cortex in the emotional and cognitive measures

Regarding the affective changes, abnormally decreased blood flow and metabolism in prefrontal cortex (including Brodmann areas 9 and 10) have been extensively replicated in neuroimaging studies of depression (Shumake and Gonzalez-Lima, 2003). These metabolic deficits, especially in right-hemisphere frontal pole regions, correlate most strongly with negative thoughts (Dunn et al., 2002). This is interesting, considering the effect of LLLT on mood is primarily reflected by the suppression of negative thoughts. The congenital helpless rat, an animal model of depression, shows metabolic suppression in prefrontal regions homologous to area 9 (Shumake et al., 2000), as well as impaired attention (Lee and Maier, 1988), suggesting that diminished functioning of this prefrontal cortical region may be an underlying cause.

Regarding the sustained attention tasks, right-hemisphere frontopolar cortical regions are commonly engaged in sustained attention (Marklund et al., 2007); activation of these right-hemisphere frontal regions seems to reflect general attention/vigilance. Good performance on a sustained attention task is correlated with enhanced activation in predominantly right-hemisphere frontal and parietal regions (Lawrence et al., 2003). The middle frontal gyrus is activated in an attention-demanding target detection task (Yamasaki et al., 2002). During attentional processes, frontal cortical regions seem to exert top-down control over noradrenergic activation from the brainstem (Robbins, 1984).

In terms of potential brain mechanisms, it has been hypothesized that during passive, baseline cognitive conditions, a ‘‘default mode’’ brain network (Raichle et al., 2001) is online in the human brain, and this network consists of a set of regions that are found in neuroimaging studies to be more active during passive tasks than in active or experimental tasks. This default mode, which is active when the individual is not particularly cognitively challenged, must be inhibited when the individual focuses attention on a specific task. Cognitive resources must be shifted away from the default mode, and instead assigned to the brain regions that are needed to focus on the new, cognitively-demanding task. During the PVT, this shift has been observed with fMRI, which showed the involvement of a frontoparietal network associated with faster reaction times and greater sustained attention in the PVT (Drummond et al., 2005). Increases in regional cerebral blood flow in right-hemisphere prefrontal and parietal regions are also associated with performance in the DMS task (Grady et al., 1998), with prefrontal cortex mediating convergent processes for increasing the accuracy of visuospatial memory during the delay portion of the DMS task (Sawaguchi and Yamane, 1999). This network, and specifically the frontal regions that likely mediate it, may be the mechanism by which the LLLT manifests the effects seen here. By augmenting the neural metabolism of the relevant frontal regions, the function of this sustained-attention network is improved, leading to better performance on the PVT and DMS. Specifically, the right middle frontal gyrus targeted by the LLLT shows the most consistent effects in supporting sustained attention (Sturm and Willmes, 2001; Lawrence et al., 2003; Drummond et al., 2005).

The three-way interaction between the trait of novelty-seeking, treatment group, and reaction time in the PVT indicates that stable personality traits may play a role in certain treatment responses correlated with the frontal cortex. The high novelty-seekers seem to be the subjects that benefit the most from the LLLT in the sustained attention PVT task. When treated with LLLT, high novelty-seekers do not just maintain the same reaction times; rather, they actually improve over their baseline performance. This is consistent with MRI findings showing that frontal cortex gray matter volume correlates positively with novelty-seeking, as measured with the TPQ test used in our study (Gardini et al., 2009). Therefore, subjects classified as high novelty-seekers appear to benefit more from frontal cortex stimulation, leading to improved PVT performance, perhaps by improving frontal cortex-based sustained attention.

Due to the biological absorption and scattering of the light by the overlying skin, skull and dura, only a small fraction of the light incident to the skin can be expected to reach the frontal cortex. The effect of the skull bone’s absorption is likely greater than that of the skin, given the lack of significant interactions with skin pigmentation level. Using an increased laser power level for the LLLT may lead to larger effect sizes, though the increased heat might present a problem with rendering subjects blind to group assignment.

Collectively, these data imply that LLLT could be used as a non-invasive and efficacious approach to increase brain functions such as those related to cognitive and emotional dimensions. LLLT may also provide neuroprotection against neurological conditions which may be related to reduced oxidative energy metabolism. This research could ultimately lead to the development of non-invasive, non-pharmacologic, therapeutic, cytoprotective and performance-enhancing interventions in both healthy humans and in those in need of rehabilitation efforts under conditions where neuronal metabolism is compromised, by treating neuropsychological disorders in which metabolic dysfunction plays an underlying causal role.

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