Transcranial Photobiomodulation Prevents Anxiety and Depression *via* Changing Serotonin and Nitric Oxide Levels in Brain of Depression Model Mice: A Study of Three Different Doses of 810 nm Laser

Emad Eshaghi, MSc,[1,2] Saeed Sadigh-Eteghad, PhD,[1] Gisou Mohaddes, PhD,[1] and Seyed Hossein Rasta, PhD[1,2,3,4]*

[1]*Neurosciences Research Center (NSRC), Tabriz University of Medical Sciences, Tabriz, Iran*
[2]*Department of Medical Physics, Tabriz University of Medical Sciences, Tabriz, Iran*
[3]*Department of Medical Bioengineering, Tabriz University of Medical Sciences, Tabriz, Iran*
[4]*School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom*

Objectives: The effectiveness of transcranial photobiomodulation (TPBM) in treating anxiety and depression disorders is a demonstrated and identified issue. However, the optimum therapeutic dose and the underlying mechanism of action are not fully understood. In this study, the therapeutic effects of three different near-infrared (NIR) doses on anxiety- and depression-like behaviors as well as cerebral levels of serotonin (5-HT) and nitric oxide (NO) were evaluated in a mouse model of chronic restraint stress (CRS).

Materials and Methods: CRS procedure (3 hours/day, over 3 weeks) was performed as a typical stress model to study anxiety and depression along with laser treatment (3 times/week, over 3 weeks), which began simultaneously with CRS. A NIR diode laser (810 nm wavelength, 10 Hz) with the output power of 200 mW and power density of 4.75 W/cm^2 was implemented to deliver three different doses of 4, 8, and 16 J/cm^2 to the cerebral cortex of mice. Behavioral experiments including open field, tail suspension, and elevated plus maze tests as well as serum cortisol levels were assessed to evaluate the anti-anxiety and anti-depressive effects of NIR TPBM. The changes of 5-HT and NO levels in the prefrontal cortex (PFC) and hippocampus (Hipp) were assessed.

Results: CRS procedure induced anxiety- and depression-like behaviors, increased serum cortisol levels, decreased 5-HT and increased NO levels in the PFC and Hipp areas. NIR TPBM improved behavioral results, decreased serum cortisol levels, increased 5-HT and decreased NO concentrations in the PFC and Hipp. A dose of 8 J/cm^2 of NIR TPBM showed the maximum effects on behavioral and molecular results, while a decline was observed from the optimum effects at both lower (4 J/cm^2) and higher (16 J/cm^2) doses.

Conclusion: Our results demonstrated that NIR TPBM had an anti-anxiety and anti-depressive effect in CRS mice, which is probably linked to increasing 5-HT and decreasing NO levels in the PFC and Hipp areas. Also, the maximum anti-anxiety and anti-depressive effect was produced at dose of 8 J/cm^2. Lasers Surg. Med. © 2019 Wiley Periodicals, Inc.

Key words: transcranial photobiomodulation; transcranial low-level light/laser therapy; depression; anxiety; serotonin; nitric oxide; near-infrared; dose-response

INTRODUCTION

Serotonin (5-HT) is considered to play a vital role in the physiopathology of anxiety, mood disorders, and depression [1]. The monoamine-deficiency hypothesis proposes that depression is resulted as a consequence of imbalance and deficiency of depression-dependent monoamine neurotransmitters including 5-HT, dopamine, and noradrenaline in the central nervous system (CNS) [2].

Nitric oxide (NO) is another important neurotransmitter, the role of which in depression, anxiety, and cognition has been reported. This gas neurotransmitter has mediatory and modulatory effects on physiological processes in the CNS, including neurotransmission, neurogenesis, synaptic plasticity, and neuroinflammation [3,4]. In addition, the decreased levels of 5-HT in the CNS are considered to be associated with an increase in NO levels. A number of studies indicated that NO synthesis (NOS) inhibitor drugs are effective in depression treatment and

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Contract grant sponsor: Tabriz University of Medical Sciences; Contract grant number: 5/88/1782.

*Correspondence to: Seyed Hossein Rasta, PhD, Department of Medical Physics and Medical Bioengineering, Tabriz University of Medical Sciences, Tabriz 5166614766, Iran. E-mail: s.h.rasta@abdn.ac.uk

Accepted 20 February 2019
Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/lsm.23082

can modify the levels of depression-dependent neurotransmitters such as 5-HT in the CNS [5,6].

Pharmacotherapy is an effective and common clinical intervention used for depression disorders. However, some disadvantages such as delayed efficacy in onset, side effects [7], and drug-resistant depression [8] are the negative implications of the approach. These disadvantages have made the search for non-pharmaceutical and non-invasive methods for depression treatment a priority.

Transcranial photobiomodulation (TPBM) or transcranial low-level light (TLLL) therapy is a non-invasive approach that has shown therapeutic benefits for neurodegenerative disorders [9], sexual dysfunction [10], and psychological problems [11]. In this non-heating approach, brain tissue is transcranially irradiated with red and near-infrared (NIR) lights (600–1100 nm) using low-powered (1–1000 mW) light-emitting diode, laser, and diode laser devices [9]. The basic biological effects of TPBM on the molecular scale is thought to be started *via* absorption of NIR and red photons by cytochrome c oxidase (COX) enzymes of the neuronal mitochondria [12]. It is hypothesized that after photon-COX interaction, the inhibitory NO is dissociated from COX and results in enhanced mitochondrial respiration, increased adenosine triphosphate (ATP) production, and increased cellular metabolic activity [13,14].

The therapeutic effects of TPBM are dependent on some parameters such as the irradiation wavelength, continuous or pulsed mode of operation, and the delivered dose on the brain cortical surface per session [13,15,16]. The positive effects of NIR TPBM, at both continuous and 10 Hz pulsed modes, in the treatment of depression were indicated in former investigations [17,18]. In studying the effects of NIR TPBM on depression, several studies have reported the delivered dose on the cortical surface. Wu et al. [19] found that delivering a NIR dose of 1.8 J/cm^2 to the cortical surface of chronic mild stress (CMS) rat per session (16.2 J/cm^2 over entire treatment) produces significant anti-depressive effects. In a similar study by Salehpour et al. [20], a NIR dose of 1.2 J/cm^2 per session (14.4 J/cm^2 over entire treatment) was delivered to the prefrontal cortex (PFC) of CMS rat. They reported that the applied NIR dose was able to significantly decrease depression-like behaviors, however, it did not cause a significant effect on anxiety-like behaviors. Delivering an inappropriate dose, low or high, to the cortical surface may have neutral or adverse therapeutic results for the biphasic dose-response effect. However, there is limited evidence to understand the optimal dose of TPBM for the treatment of anxiety and depression. Also, the alleviating effects of TPBM on depression by changing 5-HT and NO levels in the brain are not clearly elucidated.

This study was designed to assess the anti-anxiety and anti-depressive effects of three different doses of NIR TPBM in chronic restraint stress (CRS) mice using behavioral experiments including open field, tail suspension, and elevated plus maze (EPM) tests. In addition, the levels of 5-HT and NO in the PFC and hippocampus (Hipp) areas were measured to expound the possible anti-anxiety and anti-depressive molecular mechanism of NIR TPBM.

MATERIALS AND METHODS

Animals and Study Design

Fifty-five male 8-week BALB/c mice (20–25 g) were taken from the animal house of Tabriz University of Medical Sciences (TUOMS). Animals were kept under standard laboratory conditions (12 hours light/dark cycle, 20–22°C, and 45–55% humidity) with food and water *ad libitum* during the experiments. After a week of adaptation, animals were randomly separated into five groups (11 animals per group). Mice in the control group were handled gently daily with no intervention. The CRS groups received NIR doses of 0, 4, 8, and 16 J/cm^2 and named CRS + Sham, CRS + 4NIR, CRS + 8NIR, and CRS + 16NIR, respectively. TPBM therapy was initiated simultaneously with the induction of CRS. Details of the study design, including durations of adaptation time, CRS procedure, TPBM therapy, and behavioral tests are indicated in Figure 1. All used methods in this research were approved by Committee of Ethics of TUOMS (Code number: 1396.659).

CRS Procedure

To induce a depression phenotype, mice in the CRS groups (sham and NIR laser treated groups) were subjected to daily restraint stress (3 hours/day) for 3

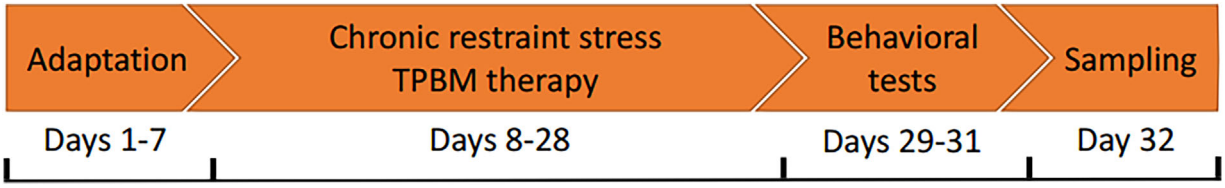

Fig. 1. Experimental design of the study. After a week of adaptation, mice underwent to CRS procedure for 3 hours/d over 3 weeks and TPBM therapy started with the onset of stress induction. Mice irradiated with three different doses (4, 8, and 16 J/cm^2) of NIR laser (810 nm, 10 Hz) for 3 times per week over 3 weeks. OFT, EPM, and TST behavioral tests were performed on 29th, 30th, and 31th days, respectively. On the last day, animals were killed and sampled from their blood and brain tissue. CRS, chronic restraint stress; TPBM, transcranial photobiomodulation; NIR, near-infrared; OFT, open field test; EPM, elevated plus maze; TST, tail suspension test.

consecutive weeks [21]. Each mouse was placed in a leaky 50 ml polypropylene cylinder (length: 100 mm; diameter: 30 mm) at certain hours in the morning (8:00–11:00) and was kept in its home cage during the rest of the day with free access to water and food.

TPBM Therapy

For TPBM, a diode laser device (GaAlAs, Class 3B) with 810 nm wavelength, 10 Hz mode of operation, 88% duty cycle, 0.0364 cm^2 spot size area, 200 mW output power, and 4.75 W/cm^2 power density was implemented. Laser irradiation was administered for 3 consecutive weeks and 3 sessions a week on odd days [19]. During exposure, the mouse was restrained manually and the laser probe was kept constant over the midline of the dorsal surface of the head in the region between the ears and eyes. The preliminary measurements showed that an average power density of 1.6 W/cm^2 transmitted through the fur, skin, and skull to the cortical surface. The irradiation times to deliver average doses of 4, 8, and 16 J/cm^2 to the cortical surface per session were 2.5, 5, and 10 seconds, respectively. The total energy delivered to the cortical surface per session was 0.15 J for the CRS + 4NIR group, 0.29 J for the CRS + 8NIR group, and 0.58 J for the CRS + 16NIR group. TPBM was done at least 3 hours following CRS procedure on days when both CRS procedure and TPBM were administered.

Open Field Test

The open field test (OFT) is an extensively used experiment to assess locomotor activity and anxiety-like behaviors in rodents. Anxiety-like behavior is based on subjecting an animal to an unknown environment which cannot escape due to surrounding walls [22]. One hour before the test, animals were entered to the test room to get adapted. Then, mice were located in the middle of the floor of a $40 \times 40 \times 35$ cm arena with acrylic walls and black floor. All the movements were recorded for 10 min with a video camera above the arena. The total traveled distance in the arena and the whole spent time in the central zone were calculated by Ethovision tracking software (Noldus) [23].

Elevated Plus Maze

The EPM is a raised plus apparatus used to study anxiety-like behaviors in rodents [24]. It consists of five parts including two enclosed arms ($30 \times 5 \times 15$ cm), two open arms (30×5 cm), and one central platform (5×5 cm) connecting the aforesaid arms. The wooden apparatus was raised 38.5 cm above the room floor on four wooden legs. One hour before the test, mice were transferred to the test room with dim illumination and left undisturbed. After adaptation time, each mouse was placed singly in the central area of the maze with its head directed toward a closed arm. All the movements were recorded for 6 min with a video camera clinging to the ceiling. Both %OAE (the number of open arm entries per the total entries into the open and enclosed arms $\times 100$) and %OAT (the time stayed in the open arms per the time stayed in the open and enclosed arms $\times 100$) parameters were calculated by video tracking program (Ethovision, Noldus).

Tail Suspension Test

The tail suspension test (TST) was used to evaluate the anti-depressive efficacy of the treatment [25]. To minimize environmental effects, animals were placed in the experiment room for 1 hour before the test. Then, mice were hung at 50 cm altitude above the table by attaching their tails to a wooden rod using adhesive tape. All the movements were recorded for 6 min with a video camera. The immobility time was calculated by the video tracking program (Ethovision, Noldus) [26].

Serum Cortisol Measurement

One day after the end of the behavioral experiments, animals were deeply anesthetized with ip injection of ketamine (75 mg/kg) and xylazine (10 mg/kg) in the morning (9:00–11:00). Then, mice were decapitated and about 1 ml blood was collected from the trunk. To obtain serum, blood samples were placed for approximately 30 min in the refrigerator to be coagulated and then they were centrifuged at 4000 rpm for 10 min at 4°C. Serum samples were frozen (−80°C) until cortisol measurement. Serum cortisol concentration was assessed by ELISA kit (Monobind Inc., Lake Forest, CA. Product Code: 3625–300) according to the manufacturer's instructions. Cortisol levels are expressed as μg/dl.

Brain Tissue 5-HT and NO Measurements

Immediately after decapitation of mice, the brain tissues were removed and put on ice pad. The PFC and Hipp areas of brain were dissected and kept in a −80°C freezer until 5-HT and NO measurements. To prepare samples, the aforesaid parts were pulverized with liquid nitrogen and then homogenized by hand with 1 ml of cold PBS. The resultants were centrifuged at 3,000 rpm at 4°C (20 min) and stored at −80°C. Mouse 5-HT ELISA kit and mouse NO ELISA kit were provided from Hangzhou Eastbiopharm Co., Ltd. (Hangzhou, China) and measurements were assessed according to the instructions provided by ELISA kit manufacturer. The levels of 5-HT and NO are expressed as pg/mg protein and μmol/mg protein, respectively.

Statistical Analysis

Statistical analyses were performed and graphs were constructed by GraphPad Prism 6.01 (GraphPad Software, Inc., San Diego, CA). All results are shown as mean ± SEM. One-way ANOVA followed by post hoc Tukey's HSD test was used to compare the differences between the groups. To determine the association between continuous variables, the Spearman's rank correlation coefficient was utilized. The statistical significance level was demonstrated to be $P < 0.05$.

RESULTS

Open Field Test

The total traveled distance and spent time in the central zone were calculated in the OFT. Results indicated that the CRS + Sham group was significantly less willing to spend time in the central zone than the control group ($P < 0.001$) (Fig. 2A). In contrast to the CRS + Sham group, both NIR doses of 8 and 16 J/cm^2 significantly increased time spent in the center area ($P < 0.01$ and $P < 0.05$, respectively) (Fig. 2A). Moreover, there were no significant differences between the treated and non-treated groups for total distances traveled (Fig. 2B).

Elevated Plus Maze

One-way ANOVA and Tukey's post-hoc test analysis revealed that both %OAE and %OAT variables were significantly decreased in the CRS + Sham group compared to the control group ($P < 0.001$) (Fig. 3A and B, respectively). In comparison to the CRS + Sham group, % OAE was significantly increased by higher NIR doses of 8 and 16 J/cm^2 ($P < 0.001$ and $P < 0.01$, respectively) and % OAT was significantly increased by doses of 4, 8, and 16 J/cm^2 ($P < 0.01$, $P < 0.001$, and $P < 0.001$, respectively).

Tail Suspension Test

As indicated in Figure 4, the immobility time in the CRS + Sham group was significantly higher than the control group ($P < 0.001$). A significant decline in the immobility time was observed in the CRS + 8NIR and CRS + 16NIR groups when compared with the CRS + Sham group ($P < 0.001$ and $P < 0.05$, respectively).

Serum Cortisol Levels

Serum cortisol levels were significantly higher in the CRS + Sham group than in the control group ($P < 0.001$) (Fig. 5). Results revealed that all the three NIR doses

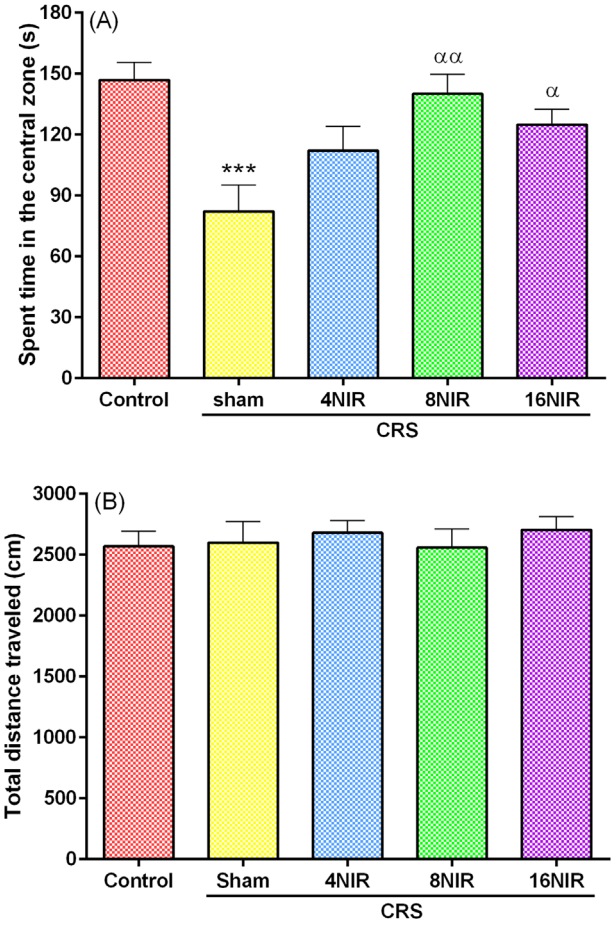

Fig. 2. The effects of three different NIR doses on (**A**) spent time in the central zone and (**B**) total distance traveled in the OFT. Data are presented as mean ± SEM ($n = 10$-11 mice). $^{***}P < 0.001$ versus control group, $^{\alpha}P < 0.05$ and $^{\alpha\alpha}P < 0.01$ versus CRS + Sham group. NIR, near-infrared; OFT, open field test; SEM, standard error of the mean; CRS, chronic restraint stress; 4NIR, a NIR dose of 4 J/cm^2 on the cortical; 8NIR, a NIR dose of 8 J/cm^2 on the cortical; 16NIR, a NIR dose of 16 J/cm^2 on the cortical.

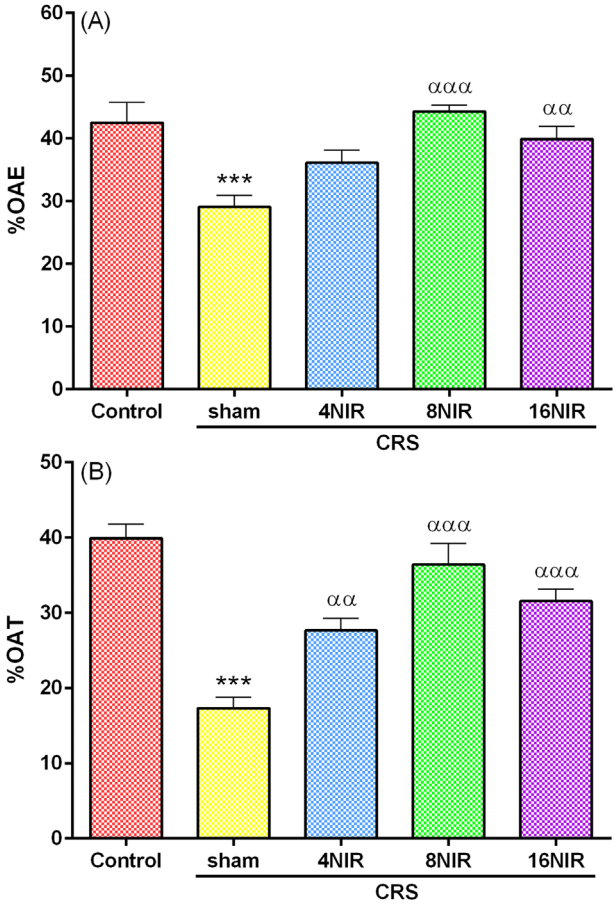

Fig. 3. The effects of three different NIR doses on (**A**) %OAE and (**B**) %OAT in the EPM. Data are presented as mean ± SEM ($n = 10$-11 mice). $^{***}P < 0.001$ versus control group, $^{\alpha\alpha}P < 0.01$ and $^{\alpha\alpha\alpha}P < 0.001$ versus CRS + Sham group. NIR, near-infrared; % OAE, percentage of open arm entries; %OAT, percentage of time stayed in the open arms; EPM, elevated plus maze; SEM, standard error of the mean; CRS, chronic restraint stress; 4NIR, a NIR dose of 4 J/cm^2 on the cortical; 8NIR, a NIR dose of 8 J/cm^2 on the cortical; 16NIR, a NIR dose of 16 J/cm^2 on the cortical.

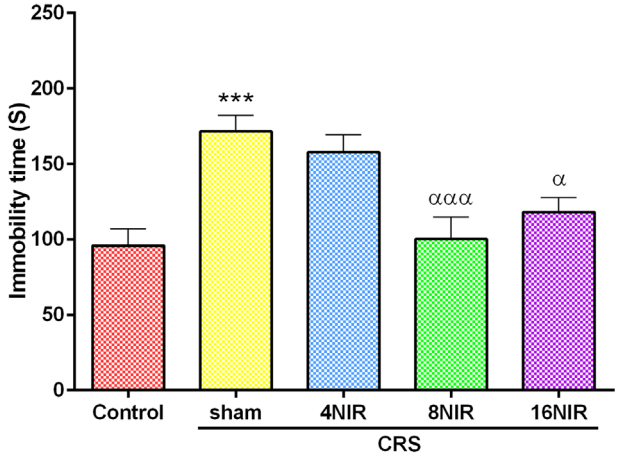

Fig. 4. The effects of three different NIR doses on immobility time in the TST. Data are presented as mean ± SEM ($n = 10$-11 mice). $^{***}P < 0.001$ versus control group, $^{\alpha}P < 0.05$ and $^{\alpha\alpha\alpha}P < 0.001$ versus CRS + Sham group. NIR, near-infrared; TST, tail suspension test; SEM, standard error of the mean; CRS, chronic restraint stress; 4NIR, a NIR dose of 4 J/cm^2 on the cortical; 8NIR, a NIR dose of 8 J/cm^2 on the cortical; 16NIR, a NIR dose of 16 J/cm^2 on the cortical.

induced a significant decrease in serum cortisol levels in comparison to the CRS + Sham group ($P < 0.001$).

Brain 5-HT Levels

As indicated in Figure 6, after the administration of CRS procedure for 3 hours/day over 21 consecutive days, the levels of 5-HT in the both PFC and Hipp areas were significantly decreased in the CRS + Sham group compared to the control group ($P < 0.01$) (Fig. 6A and B, respectively). TPBM reversed the effects of CRS and NIR doses of 8 and 16 J/cm^2 significantly increased the levels of

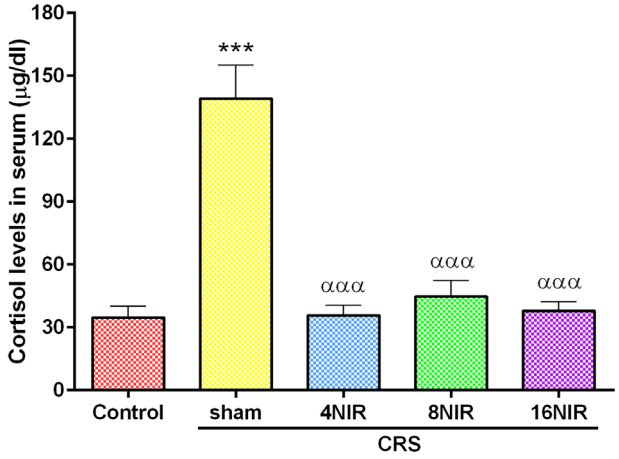

Fig. 5. The effects of three different NIR doses on blood cortisol levels. Data are presented as mean ± SEM ($n = 10$-11). $^{***}P < 0.001$ versus control group, $^{\alpha\alpha\alpha}P < 0.001$ versus CRS + Sham group. NIR, near-infrared; SEM, standard error of the mean; CRS, chronic restraint stress; 4NIR, a NIR dose of 4 J/cm^2 on the cortical; 8NIR, a NIR dose of 8 J/cm^2 on the cortical; 16NIR, a NIR dose of 16 J/cm^2 on the cortical.

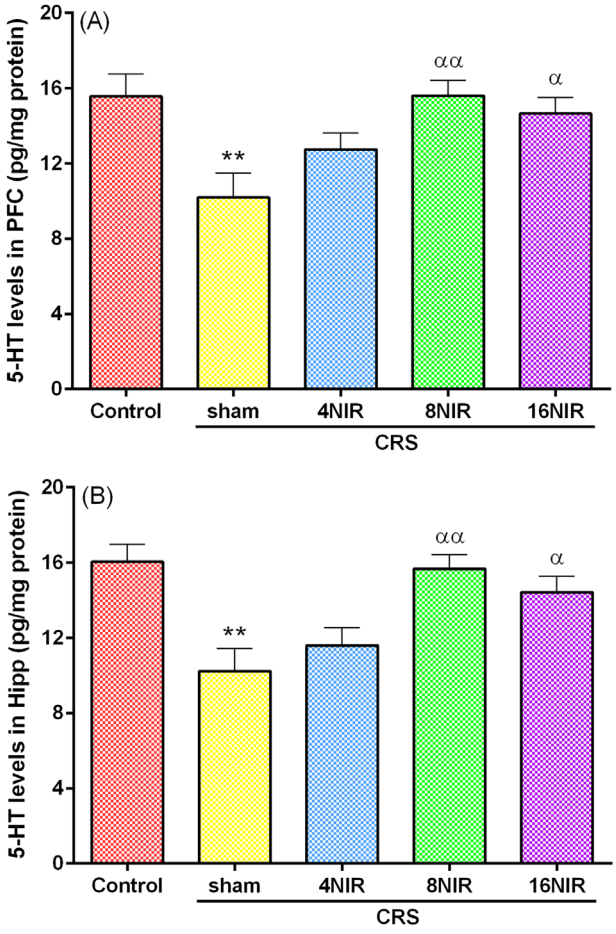

Fig. 6. The effects of three different NIR doses on 5-HT levels in the (**A**) PFC and (**B**) Hipp areas. Data are presented as mean ± SEM ($n = 6$-7). $^{**}P < 0.01$ versus control group, $^{\alpha}P < 0.05$ and $^{\alpha\alpha}P < 0.01$ versus CRS + Sham group. NIR, near-infrared; 5-HT, serotonin; PFC, prefrontal cortex; Hipp, hippocampus; SEM, standard error of the mean; CRS, chronic restraint stress; 4NIR, a NIR dose of 4 J/cm^2 on the cortical; 8NIR, a NIR dose of 8 J/cm^2 on the cortical; 16NIR, a NIR dose of 16 J/cm^2 on the cortical.

5-HT in the PFC ($P < 0.01$ and $P < 0.05$, respectively) and Hipp ($P < 0.01$ and $P < 0.05$, respectively) areas compared to the CRS + Sham group.

Association Between Immobility Time and 5-HT Levels

To investigate if depression-like behavior might be related with brain 5-HT levels, the associations between immobility time in the TST and 5-HT levels in the both PFC and Hipp areas were measured. As depicted in Figure 7, the immobility time was significantly and negatively correlated to the 5-HT levels. The correlation coefficients for PFC and Hipp were −0.87 ($P < 0.001$) (Fig. 7A) and −0.85 ($P < 0.001$) (Fig. 7B), respectively.

Brain NO Levels

The levels of NO in the PFC and Hipp areas were significantly increased in the CRS + Sham group compared

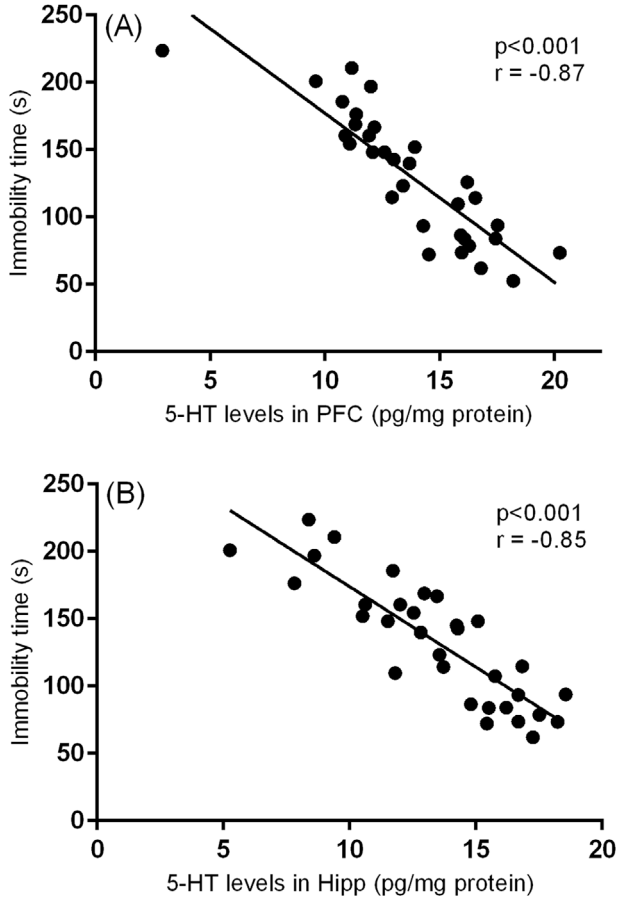

Fig. 7. Association between immobility time in the TST and 5-HT levels in the (**A**) PFC and (**B**) Hipp areas. The correlation coefficients for the PFC and Hipp were −0.87 ($P < 0.001$) and −0.85 ($P < 0.001$), respectively. TST, tail suspension test; 5-HT, serotonin; PFC, prefrontal cortex; Hipp, hippocampus.

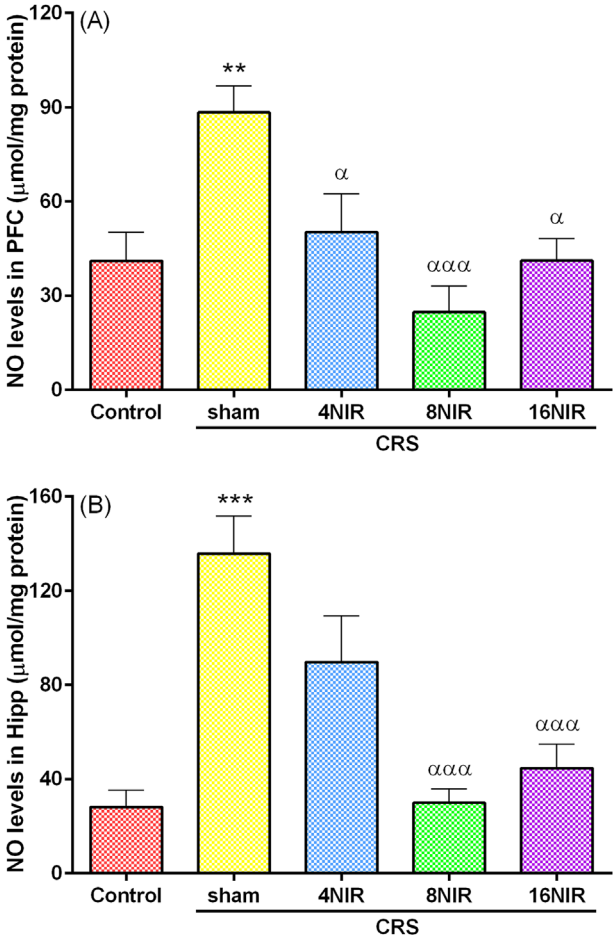

Fig. 8. The effects of three different NIR doses on NO levels in the (**A**) PFC and (**B**) Hipp areas. Data are presented as mean ± SEM ($n = 6$-8). $^{**}P < 0.01$ and $^{***}P < 0.001$ versus control group, $^{\alpha}P < 0.05$ and $^{\alpha\alpha\alpha}P < 0.001$ versus CRS + Sham group. NIR, near-infrared; NO, nitric oxide; PFC, prefrontal cortex; Hipp, hippocampus; SEM, standard error of the mean; CRS, chronic restraint stress; 4NIR, a NIR dose of 4 J/cm^2 on the cortical; 8NIR, a NIR dose of 8 J/cm^2 on the cortical; 16NIR, a NIR dose of 16 J/cm^2 on the cortical.

to the control group ($P < 0.01$ and $P < 0.001$, respectively) (Fig. 8A and B, respectively). In comparison to the CRS + Sham group, NO levels were significantly decreased in the PFC by NIR doses of 4, 8, and 16 J/cm^2 ($P < 0.05$, $P < 0.001$, and $P < 0.05$, respectively), while they were significantly decreased in the Hipp by higher doses of 8 and 16 J/cm^2 ($P < 0.001$).

Association Between NO and 5-HT Levels

The correlations between NO and 5-HT concentrations in the PFC and Hipp areas of mice brain were analyzed. NO concentrations had a significant and strong negative correlation with 5-HT levels. The correlation coefficients for PFC and Hipp were −0.88 ($P < 0.001$) (Fig. 9A) and −0.89 ($P < 0.001$) (Fig. 9B), respectively.

DISCUSSION

This study was designed to evaluate the effects of three different doses of NIR TPBM on anxiety- and depression-like behaviors and the levels of depression-dependent neurotransmitters, 5-HT and NO, in the PFC and Hipp areas in CRS mice. CRS procedure was used as a common stress model to study anxiety- and depression-like behaviors and to examine the pathophysiology of depression. Here, the exposure to CRS procedure mimicked anxiety and depression status by decreasing time spent in the central zone in the OFT, reducing %OAE and %OAT in the EPM, and rising immobility time in the TST and serum cortisol levels, along with changes in the PFC and Hipp areas, the decreasing of 5-HT and increasing of NO levels. However, NIR TPBM reversed the anxiety and depression status caused by CRS procedure that was probably linked to increased 5-HT and decreased NO levels in the both PFC and Hipp areas. The effects of NIR TPBM were dose-dependent and the maximum anti-anxiety and anti-depressive benefits induced by 8 J/cm^2, whereas the benefits declined at lower (4 J/cm^2) and higher (16 J/cm^2) doses.

Administration of NIR TPBM with a dose of 8 J/cm^2 induced the maximum incremental effect on spent time

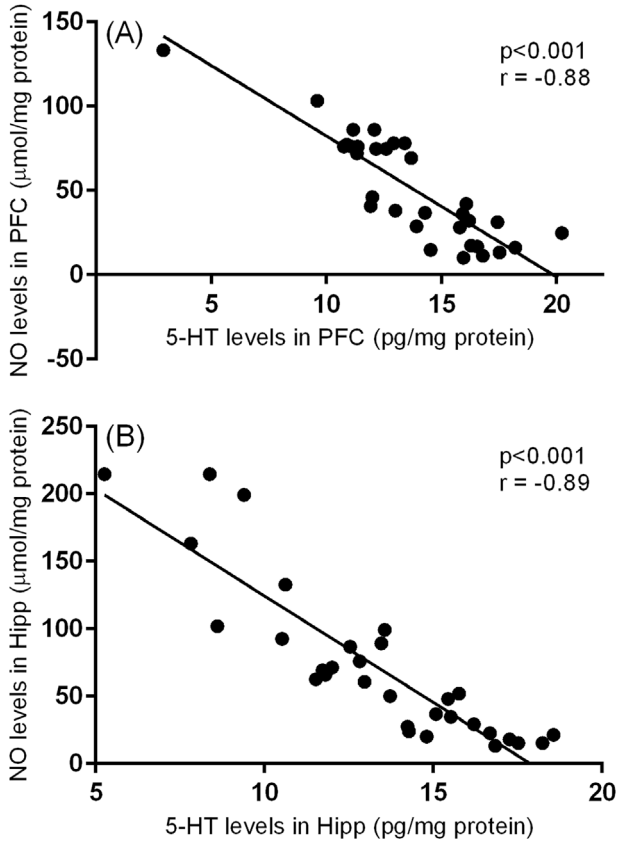

Fig. 9. Association between the levels of NO and 5-HT in the (**A**) PFC and (**B**) Hipp areas. The correlation coefficients for the PFC and Hipp were -0.88 ($P < 0.001$) and -0.89 ($P < 0.001$), respectively. NO, nitric oxide; 5-HT, serotonin; PFC, prefrontal cortex; Hipp, hippocampus.

in the central zone in the OFT, even better than 16 J/cm^2, and the lowest applied NIR dose (4 J/cm^2) caused the minimum anti-anxiety benefit. On the other hand, these results indicated an inverted U-shaped dose-response effect, with the maximum anti-anxiety benefit at dose of 8 J/cm^2.

In the EPM test, the CRS + Sham group which exposed to CRS procedure was less tended to enter and spent time in the open arms and both %OAE and %OAT parameters significantly declined compared to the control group. These results are parallel to the prior depression studies [27,28]. In a study by Salehpour et al. [20] the anti-anxiety effect of NIR (810 nm, 10 Hz) TPBM in CMS rat was evaluated by EPM test. In their study, a NIR dose of 1.2 J/cm^2 was delivered to the PFC and non-significant improvements in the both %OAE and %OAT variables were observed. In the current study, three higher doses were applied and expanded areas of brain were irradiated. It was observed that all the three applied doses reduced anxiety-like behaviors and both %OAE and %OAT were significantly improved compared with the CRS + Sham group. The anti-anxiety effects of three applied doses followed an inverted U-shaped dose-response effect, with the maximum anti-anxiety benefit at dose of 8 J/cm^2.

In the TST test, the CRS + Sham group showed significant increasing immobility time that confirms the results of a previous depression study [21]. However, NIR TPBM at higher doses (8 and 16 J/cm^2) significantly attenuated immobility time compared with the CRS + Sham group. Our results showed a U-shaped dose-response effect, with the minimum depression-like behavior at dose of 8 J/cm^2. The anti-depressive effect of NIR TPBM on TST in CRS mice was also reported by Xu et al. [29]. In their study, mice were irradiated (808 nm, 23 mW/cm^2) over 28 consecutive days, once per day, and a dose of 41.4 J/cm^2 was delivered on the shaved scalp at each therapeutic session. In another study, Ando et al. [30] observed an anti-depressive effect of pulsed NIR laser (810 nm, 10 Hz) at dose of 36 J/cm^2, on the scalp, in traumatic brain injury mice by TST. It should be noted that in former studies, delivering NIR doses of 1.2 J/cm^2 to the PFC [20] and 1.8 J/cm^2 to the cortical surface [19] of CMS rat per session caused significant anti-depressive effects in the force swimming test (FST). In comparison, in this study, a higher delivered NIR dose of 4 J/cm^2 to the cerebral surface of CRS mice per session did not make a significant anti-depressive effect in the TST. These evidences suggest that low doses of NIR TPBM lead to anti-depressive effects in the FST but not in the TST.

Serum cortisol levels were significantly increased in mice underwent to CRS procedure that confirms the results of a previous depression study [31]. The effect of NIR (810 nm, 10 Hz) TPBM on cortisol levels in CMS rat model was examined by Salehpour et al. [20] and no significant changes were observed in cortisol concentrations after TPBM. Probably, the delivered dose to the PFC (about 1.2 J/cm^2) was not sufficient to make a therapeutic effect. In comparison, the current study delivered three higher doses to the expanded cerebral cortex, which resulted in significant reduced cortisol levels.

Decreased 5-HT levels in the PFC and Hipp areas have been demonstrated in previous animal depression studies [32,33], which is in agreement with this study. Few studies have investigated the effects of TPBM on 5-HT levels in the brain tissue. Cassone et al. [34] evaluated the effects of He-Ne laser (632.8 nm) on 5-HT concentrations in the brain areas of stressed rat. Nine spots on the sinciput were irradiated and a dose of 1.08 J/cm^2 was administered per animal. It was observed that, 5-HT levels significantly increased in the Hipp and striatum, however, they insignificantly altered in the cortex. In this study, NIR TPBM at doses of 8 and 16 J/cm^2 significantly increased 5-HT levels in the PFC and Hipp areas, which is compatible with the Hipp data of the Cassone study, but not cortex. One probable reason is that this study specifically measured 5-HT in the PFC, whereas in Cassone et al. study the measurement was performed in the cortex and was not on a specific area. The effects of three different NIR doses on 5-HT levels in the both PFC and Hipp areas were similar and an inverted U-shaped dose-response effect was observed. The most incremental effects on 5-HT concentrations were resulted at a dose of 8 J/cm^2, whereas they were declined from the peak point at lower (4 J/cm^2) and

higher (16 J/cm^2) doses. According to the monoamine-deficiency hypothesis, imbalanced levels and noticeable decline of monoamine neurotransmitters in the brain, such as 5-HT, are considered as the main cause of depression and are associated to depression-like behaviors in animals [2]. In this study, strong negative correlations between immobility time in the TST and 5-HT levels in the both PFC and Hipp areas were observed, which in line with the monoamine-deficiency hypothesis.

A body of studies on depressed patients and animals have found that NO levels were significantly increased in the PFC and different hippocampal regions [5,35,36]. Similarly, in this study, significant higher NO levels in the PFC and Hipp areas in mice exposed to CRS procedure were found. Decreasing the levels of NO or blocking NOS in brain have been reported to cause anti-depressive effects [36–38]. A number of studies have indicated that laser irradiation significantly decreased the synthesis and levels of NO. Leung et al. [39] delivered three different doses (2.64, 13.2, and 26.4 J/cm^2) of GaAlAr laser (660 nm, 10 kHz) on the ischemic rat cerebral cortex and measured the specific activity of NOS and expression of inducible NOS (iNOS), neuronal NOS (nNOS), and endothelial NOS (eNOS) isoforms. It was observed that all the three applied doses significantly suppressed NOS activity and expression of all NOS isoforms. Gonçalves et al. [40] employed a multiple sclerosis (MS) mouse model and irradiated the spinal cord with AlGaInP (660 nm, 10 J/cm^2) and GaAs (904 nm, 3 J/cm^2) diode lasers. Their study demonstrated that both the applied doses significantly decreased NO concentrations in the spinal cord. In research on treatment of rat's injured sciatic nerve, Gomes et al. [41] found that HeNe laser irradiation at dose of 10 J/cm^2 significantly suppressed iNOS expression. In this study, it was observed that NIR TPBM mimicked NOS inhibitor action and NO levels were significantly declined in the both PFC and Hipp areas. The maximum reduction in NO levels was observed at dose of 8 J/cm^2, whereas an increase from the minimum NO levels was seen at two higher (16 J/cm^2) and lower (4 J/cm^2) doses. Other words, the inhibitory effects of NIR TPBM on NO levels were dose-dependent and followed a U-shaped dose-response effect, with the maximum reduction at dose of 8 J/cm^2. It should be mentioned that low levels of NO are neuroprotective whereas higher levels are responsible for inflammation and toxicity. The reason is that high amounts of NO react with superoxide (O_2^-) free radicals and produce peroxynitrite anions ($ONOO^-$). These reactive nitrite species are neurotoxic and lead to mitochondrial respiratory dysfunction and DNA damage [42]. Moreover, enhanced productions of NO trigger soluble guanylate cyclase, which has the responsibility for converting guanosine triphosphate into cyclic guanosine monophosphate (cGMP). Several studies on animal models have demonstrated that extreme levels of cGMP cause a depression-like state [35,43].

The relation between NO and 5-HT levels has been examined in a body of studies. Wegener et al. [44] evaluated the effects of non-selective and selective NOS inhibitor drugs on the extracellular amounts of 5-HT in the rat ventral Hipp. They observed that administration of NOS inhibitor drugs significantly increased extracellular amounts of 5-HT. Also, some other studies have indicated similar results of NOS inhibitor drugs in the hypothalamus [45], raphe nuclei, and frontal cortex [46]. It might be suggested that increased levels of 5-HT in the brain tissue are related to decreased brain levels of NO. In the current study, a strong negative correlation was observed between NO and 5-HT levels in the both PFC and Hipp areas. The inhibitory effects of NIR TPBM on NO levels were observed for all the three applied doses, however, NIR TPBM at dose of 8 J/cm^2 resulted the best consequence. This explains why the most incremental effects on 5-HT concentrations were seen at dose of 8 J/cm^2, not at higher (16 J/cm^2) or lower (4 J/cm^2) doses. Briefly, the probable mechanism is that elevated NO concentrations increase pro-inflammation cytokines that in turn enhance the activity of indoleamine 2, 3-dioxygenase enzyme. This enzyme break-downs tryptophan, through kynurenic acid pathway, which is a necessary precursor for 5-HT synthesis [35,47].

CONCLUSION

This study indicated that the anti-anxiety and anti-depressive effects of NIR TPBM are dependent on the delivered dose on the cortical surface of the brain. NIR TPBM at dose of 8 J/cm^2 caused the maximum anti-anxiety and anti-depressive effects, whereas the benefits declined at lower (4 J/cm^2) and higher (16 J/cm^2) doses. Also, it was found that the anti-anxiety and anti-depressive mechanism of NIR TPBM in mice model of CRS is probably related to increasing 5-HT and decreasing NO levels in the PFC and Hipp areas of brain.

ACKNOWLEDGMENTS

This study was financially supported by the Neurosciences Research Center (NSRC) of Tabriz University of Medical Sciences (Grant No: 5/88/1782). The article is derived from the MSc dissertation of Mr. Emad Eshaghi.

REFERENCES

1. Glover ME, Clinton SM. Of rodents and humans: A comparative review of the neurobehavioral effects of early life SSRI exposure in preclinical and clinical research. Int J Dev Neurosci 2016;51:50–72.
2. Jesulola E, Micalos P, Baguley IJ. Understanding the pathophysiology of depression: From monoamines to the neurogenesis hypothesis model—Are we there yet? Behav Brain Res 2018;341:79–90.
3. Esplugues JV. NO as a signalling molecule in the nervous system. Br J Pharmacol 2002;135(5):1079–1095.
4. Chong CM, Ai N, Ke M, et al. Roles of nitric oxide synthase isoforms in neurogenesis. Mol Neurobiol 2018;55(3):2645–2652.
5. Dhir A, Kulkarni SK. Nitric oxide and major depression. Nitric Oxide 2011;24(3):125–131.
6. Maia-de-Oliveira JP, Kandratavicius L, Nunes EA, Machado-de-Sousa JP, Hallak JE, Dursun SM. Nitric oxide's involvement in the spectrum of psychotic disorders. Curr Med Chem 2016;23(24):2680–2691.
7. Shankman SA, Gorka SM, Katz AC, et al. Side effects to antidepressant treatment in patients with depression and comorbid panic disorder. J Clin Psychiatry 2017;78(4):433–440.
8. Stimpson N, Agrawal N, Lewis G. Randomised controlled trials investigating pharmacological and psychological interventions

for treatment-refractory depression. Systematic review. Br J Psychiatry 2002;181:284–294.
9. Salehpour F, Mahmoudi J, Kamari F, Sadigh-Eteghad S, Rasta SH, Hamblin MR. Brain photobiomodulation therapy: A narrative review. Mol Neurobiol 2018;55(8):6601–6636.
10. Cassano P, Dording C, Thomas G, et al. Effects of transcranial photobiomodulation with near-infrared light on sexual dysfunction. Lasers Surg Med 2019;51(2):127–135.
11. Cassano P, Petrie SR, Hamblin MR, Henderson TA, Iosifescu DV. Review of transcranial photobiomodulation for major depressive disorder: targeting brain metabolism, inflammation, oxidative stress, and neurogenesis. Neurophotonics 2016;3(3):031404.
12. Karu TI. Cellular and molecular mechanisms of photobiomodulation (low-power laser therapy). IEEE J Selected Topics Quantum Electron 2014;20(2):143–148.
13. Hennessy M, Hamblin MR. Photobiomodulation and the brain: A new paradigm. J Opt 2017;19(1):013003.
14. Mignon C, Uzunbajakava NE, Castellano-Pellicena I, Botchkareva NV, Tobin DJ. Differential response of human dermal fibroblast subpopulations to visible and near-infrared light: Potential of photobiomodulation for addressing cutaneous conditions. Lasers Surg Med 2018;50(8):859–882.
15. Souza-Barros L, Dhaidan G, Maunula M, et al. Skin color and tissue thickness effects on transmittance, reflectance, and skin temperature when using 635 and 808nm lasers in low intensity therapeutics. Lasers Surg Med 2018;50(4):291–301.
16. Hashmi JT, Huang YY, Sharma SK, et al. Effect of pulsing in low-level light therapy. Lasers Surg Med 2010;42(6):450–466.
17. Salehpour F, Rasta SH. The potential of transcranial photobiomodulation therapy for treatment of major depressive disorder. Rev Neurosci 2017;28(4):441–453.
18. Caldieraro MA, Cassano P. Transcranial and systemic photobiomodulation for major depressive disorder: A systematic review of efficacy, tolerability and biological mechanisms. J Affect Disord 2019;243:262–273.
19. Wu X, Alberico SL, Moges H, De Taboada L, Tedford CE, Anders JJ. Pulsed light irradiation improves behavioral outcome in a rat model of chronic mild stress. Lasers Surg Med 2012;44(3):227–232.
20. Salehpour F, Rasta SH, Mohaddes G, Sadigh-Eteghad S, Salarirad S. Therapeutic effects of 10-HzPulsed wave lasers in rat depression model: A comparison between near-infrared and red wavelengths. Lasers Surg Med 2016;48(7):695–705.
21. Ding Q, Li H, Tian X, et al. Zinc and imipramine reverse the depression-like behavior in mice induced by chronic restraint stress. J Affect Disord 2016;197:100–106.
22. Walsh RN, Cummins RA. The open-field test: A critical review. Psychol Bull 1976;83(3):482–504.
23. Eilam D. Open-field behavior withstands drastic changes in arena size. Behav Brain Res 2003;142(1–2):53–62.
24. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl) 1987;92(2):180–185.
25. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology (Berl) 1985;85(3):367–370.
26. Chmielarz P, Kusmierczyk J, Parlato R, Schutz G, Nalepa I, Kreiner G. Inactivation of glucocorticoid receptor in noradrenergic system influences anxiety- and depressive-like behavior in mice. PLoS ONE 2013;8(8):e72632.
27. Carneiro de Oliveira PE, Zaniboni CR, Carmona IM, Fonseca AR, Canto-de-Souza A. Preliminary behavioral assessment of cagemates living with conspecifics submitted to chronic restraint stress in mice. Neurosci Lett 2017;657:204–210.
28. Dong L, Wang S, Li Y, et al. RU486 reverses emotional disorders by influencing astrocytes and endoplasmic reticulum stress in chronic restraint stress challenged rats. Cell Physiol Biochem 2017;42(3):1098–1108.
29. Xu Z, Guo X, Yang Y, et al. Low-level laser irradiation improves depression-like behaviors in mice. Mol Neurobiol 2017;54(6):4551–4559.
30. Ando T, Xuan W, Xu T, et al. Comparison of therapeutic effects between pulsed and continuous wave 810-nm wavelength laser irradiation for traumatic brain injury in mice. PLoS ONE 2011;6(10):e26212.
31. Liu Y, Zhuang X, Gou L, et al. Protective effects of nizofenone administration on the cognitive impairments induced by chronic restraint stress in mice. Pharmacol Biochem Behav 2013;103(3):474–480.
32. Sun J, Wang F, Hong G, et al. Antidepressant-like effects of sodium butyrate and its possible mechanisms of action in mice exposed to chronic unpredictable mild stress. Neurosci Lett 2016;618:159–166.
33. Tianzhu Z, Shihai Y, Juan D. Antidepressant-like effects of cordycepin in a mice model of chronic unpredictable mild stress. Evid Based Complement Alternat Med 2014;2014:438506.
34. Cassone MC, Lombard A, Rossetti V, Urciuoli R, Rolfo PM. Effect of *in vivo* He-Ne laser irradiation on biogenic amine levels in rat brain. J Photochem Photobiol B 1993;18(2–3):291–294.
35. Kudlow P, Cha DS, Carvalho AF, McIntyre RS. Nitric oxide and major depressive disorder: Pathophysiology and treatment implications. Curr Mol Med 2016;16(2):206–215.
36. Pu R, Shao RH, Lu J, Jin SY, Tu Y. [Acupuncture alleviated depressive-like behavior by down-regulating NF-kappaB, iNOS and NO levels in prefrontal cortex of depression rats]. Zhen Ci Yan Jiu 2018;43(4):226–230.
37. Gigliucci V, Gormley S, Gibney S, et al. Characterisation of the antidepressant properties of nitric oxide synthase inhibitors in the olfactory bulbectomised rat model of depression. European Neuropsychopharmacology 2014;24(8):1349–1361.
38. Tomaz VS, Cordeiro RC, Costa AMN, et al. Antidepressant-like effect of nitric oxide synthase inhibitors and sildenafil against lipopolysaccharide-induced depressive-like behavior in mice. Neuroscience 2014;268:236–246.
39. Leung MC, Lo SC, Siu FK, So KF. Treatment of experimentally induced transient cerebral ischemia with low energy laser inhibits nitric oxide synthase activity and up-regulates the expression of transforming growth factor-beta 1. Lasers Surg Med 2002;31(4):283–288.
40. Goncalves ED, Souza PS, Lieberknecht V, et al. Low-level laser therapy ameliorates disease progression in a mouse model of multiple sclerosis. Autoimmunity 2016;49(2):132–142.
41. Gomes LE, Dalmarco EM, Andre ES. The brain-derived neurotrophic factor, nerve growth factor, neurotrophin-3, and induced nitric oxide synthase expressions after low-level laser therapy in an axonotmesis experimental model. Photomed Laser Surg 2012;30(11):642–647.
42. Ghasemi M, Mayasi Y, Hannoun A, Eslami SM, Carandang R. Nitric oxide and mitochondrial function in neurological diseases. Neuroscience 2018;376:48–71.
43. Ben Aissa M, Lee SH, Bennett BM, Thatcher GR. Targeting NO/cGMP signaling in the CNS for neurodegeneration and Alzheimer's disease. Curr Med Chem 2016;23(24):2770–2788.
44. Wegener G, Volke V, Rosenberg R. Endogenous nitric oxide decreases hippocampal levels of serotonin and dopamine in vivo. Br J Pharmacol 2000;130(3):575–580.
45. Kaehler ST, Singewald N, Sinner C, Philippu A. Nitric oxide modulates the release of serotonin in the rat hypothalamus. Brain Res 1999;835(2):346–349.
46. Smith JC, Whitton PS. Nitric oxide modulates N-methyl-D-aspartate-evoked serotonin release in the raphe nuclei and frontal cortex of the freely moving rat. Neurosci Lett 2000;291(1):5–8.
47. Wigner P, Czarny P, Galecki P, Su KP, Sliwinski T. The molecular aspects of oxidative & nitrosative stress and the tryptophan catabolites pathway (TRYCATs) as potential causes of depression. Psychiatry Res 2018;262:566–574.