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ORIGINAL RESEARCH

# Improved Spatial Memory And Neuroinflammatory Profile Changes in Aged Rats Submitted to Photobiomodulation Therapy

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## Abstract

Recent evidences have shown the therapeutic potential of transcranial photobiomodulation on traumatic brain injury and Alzheimer's disease. Despite the promising benefits in the brain, little is known about the laser's effects in the absence of pathological conditions. We submitted young (4 months old) and aged (20 months old) rats to transcranial low-level laser and evaluated their exploratory activity and habituation in open field, anxiety in elevated plus maze, spatial memory in Barnes maze, and aversive memory in a step-down inhibitory avoidance task. Additionally, the levels of a panel of inflammatory cytokines and chemokines were quantified in two different brain regions: the cerebral cortex and the hippocampus. Young and aged rats submitted to transcranial laser exhibited better cognitive performance in Barnes maze than did control rats. Transcranial laser therapy decreased cortical levels of GM-CSF, IL-10, MCP-1, LIX, and TNFα in young rats and IL-5 in aged rats. High levels of IL-6, IL-10, and TNF-alpha were found in the cerebral cortex of aged rats submitted to transcranial laser. In the hippocampus, a decrease in IP-10 and fractalkine levels was observed in the aged rats from the laser group when compared to the aged rats from the control group. Our data indicate that transcranial photobiomodulation improves spatial learning and memory and alters the neuroinflammatory profile of young and aged rats' brains.

**Keywords** Photobiomodulation · Brain · Aging · Cognitive function · Learning · Cytokine

## Introduction

Aging is a progressive multifactorial process associated with cognitive function impairment and increased susceptibility to neurodegenerative diseases (Mattson and Magnus 2006). During aging, immune function is attenuated (immunosenescence) (Castle 2000) and the activation of immune system cells triggers a more reactive phenotype, increasing the activation of neuroinflammatory cytokines (Godbout et al. 2005). Interestingly, an imbalance between pro- and anti-inflammatory cytokines has been observed in the aged brain with a shift toward a pro-inflammatory state (Godbout and Johnson 2009).

Currently, several non-pharmacological treatments have been proposed for the improvement of cognitive functions in the elderly, such as cognitive therapy (Olazaran et al. 2004; Rozzini et al. 2007) and physical exercise (Cardoso et al. 2017). Photobiomodulation therapy (PBMT) using lasers and LEDs has attracted the interest of the scientific community for being a non-invasive therapy, that may have the capability of promoting several

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beneficial effects, such as faster healing of lesions and reduced sensitivity to pain and inflammation in several diseases (Bjordal et al. 2006a, b; Chung et al. 2012). For instance, Albertini et al. (2004) first verified that laser therapy was effective in reducing inflammation in rat paw edema similar to the classical NSAID diclofenac. Lopes-Martins et al. (2005) noted that laser therapy was able to reduce carrageenan-induced mice pleurisy and Bjordal et al. (2006b) observed that the laser inhibited prostaglandin E2 production in patients with achilles tendinitis using a real-time microdialysis system. The first evidences for the anti-inflammatory mechanisms of PBMT were described by Lopes-Martins et al. (2006) and Marcos et al. (2011), showing that the laser inhibited cyclooxygenase–2 in inflamed tendons. A second hypothesis for the effects of lasers involves the ability of cytochrome c oxidase to absorb photons, leading to the photodissociation of inhibitory nitric oxide (NO), thus promoting an increase in mitochondrial membrane potential, oxygen consumption, and production of ATP (Chen et al. 2011; Huang et al. 2009; Hamblin 2017). These changes contribute to the induction of transcription factors, such as the nuclear factor kappa B (NF-κB), p53, activating transcription factor / cAMP responsive element binding protein (ATF/CREB), hypoxia-inducing factor (HIF)-1, which promote protein synthesis, increased proliferation, and migration of cells, thus modulating the levels of inflammatory mediators (Karu and Kolyakov 2005; Chung et al. 2012).

Recent studies have documented several beneficial effects of PBMT for neurological conditions, such as depression, traumatic brain injury, Parkinson's disease, and Alzheimer's disease (AD). Xuan et al. (2014) observed an improvement in spatial memory and learning of mice submitted to laser treatment four hours after traumatic brain injury. They also showed that PBMT stimulated neurogenesis in these mice. Cognitive performance improvement was also seen in animal models of AD accompanied by increased ATP levels, increased neuronal activation (c-fos), and decreased inflammatory markers (TNF-alpha and IL-1beta) (Taboada et al. 2011).

Despite the promising effects described above, most studies have evaluated PBMT only in neuropathological conditions (Oron et al. 2007; Taboada et al. 2011; Xuan et al. 2014). Thus, this study aimed to evaluate the neurobiological effect of PBMT on the healthy young and aged brain using a 100 mW power diode laser at 810 nm. Our hypothesis is that PBMT can improve the cognitive performance of rats in different stages of life (young and aged) and that the beneficial neurobiological effect may be related to the modulation of the neuroinflammatory profile in their cortex and hippocampus.

## Materials and Methods

### Animals

Sixty-four male Wistar rats, young (4 months old) and aged (20 months old), were used in this study. The animals were housed at a temperature of 21 ± 2 °C with a 12 h light/dark cycle (lights on from 7 am to 7 pm), and food and water were provided ad libitum throughout the experimental period. All procedures were approved by the ethics committee of the University of Mogi das Cruzes (UMC) (# 016/2017) and all effort was made to minimize animal suffering in accordance with the proposals of the International Ethical Guidelines for Biomedical Research (CIOMS 1985) (CIOMS, Council for International Organizations of Medical Sciences 1985).

### Laser Therapy Protocol

The rats were randomly distributed into four groups: young laser (YL; $n = 15$), young control (YC; $n = 15$), aged laser (AL; $n = 18$), and aged control (AC; $n = 16$). The animals from the laser groups (YL and AL) were manually immobilized and received the treatment with a laser diode of 810 nm wavelength and 100 mW power (DMC Equipment, São Carlos—Brazil) transcranially for 30 s (3 J of total energy/point) at each of the five irradiation points of application (point 1 = AP + 4.20 mm and ML 0.00 mm; point 2 = AP -3.00 mm and ML -6.60 mm; 3 = AP -3.00 mm and ML + 6.60 mm; point 4 = AP 0.00 mm and ML 0.00 mm; point 5 = AP -5.52 mm and ML 0.00 mm) (Fig. 1), totalizing 15 J of Energy, 150 s of irradiation, and fluency of 535,7 J/cm$^2$ daily, between 12:00 and 1:00 pm. The animals


**Fig. 1** Five irradiation points over the scalp (target coordinates: point 1 = AP + 4.20 mm, ML 0.00 mm; point 2 = AP − 3.00 mm, ML -6.60 mm; point 3 = AP − 3.00 mm, ML + 6.60 mm; point 4 = AP 0.00 mm, ML 0.00 mm; point 5 = AP − 5.52 mm, ML 0.00 mm)

in the control group were handled the same way except that the laser was off (placebo). Laser or placebo treatment was maintained throughout all the experiments until the animals were euthanized.

## Behavioral Analyses

After 28 consecutive sessions of PBMT or placebo, the rats were submitted to behavioral tests performed sequentially in the following order: open field test, Barnes maze, elevated plus maze, and inhibitory avoidance. All behavioral procedures were conducted between 2:00 and 5:00 pm in a soundproof room. The behavioral analyses were performed independently by two investigators.

Thus, we had in the present study: 28 days of previous treatment with laser or placebo + 16 days of tests + five days of rest (four days between the open field and Barnes maze and one day between the elevated plus maze and inhibitory avoidance) + nine days of behavioral washout (in order to "wash out" the brain of the effects of behavioral tests) (Fig. 2).

### Open Field Test

The open field test was used to evaluate both exploratory activity and habituation. The apparatus consisted of a white circular arena (100 cm in diameter) made of acrylic polyvinyl. The floor of the arena was divided into 12 quadrants of equal area by lines. The animal was gently placed in the center of the arena to explore for 5 min. The rats were returned to their home cage immediately after the experiment finished. Crossings of quadrant lines were counted and used as measures of locomotion and exploration. To evaluate habituation, the experiment was repeated for three consecutive days. A decrease in the number of crossings throughout the three days of test indicates that the animal has been habituated, a type of non-associative memory (Vianna et al. 2000).

### Barnes Maze

Learning and spatial memory was tested in a Barnes maze. The Barnes maze consists of a circular platform (1.22 m diameter) with 18 evenly spaced holes (9.5 cm diameter) at the circumference edge. Only one of the holes leads to an escape box (18 cm diameter) under the maze. Objects (pictures, frames, figures) were placed on the walls of the room to be used as clues. The animals were tested once a day for ten consecutive days and allowed to freely explore the maze for up to two minutes to locate the escape box. If the animal did not find the escape box, it was gently guided to the correct hole. The latency to escape was measured. Memory is reflected in a decrease of this latency throughout training.

### Elevated Plus Maze

Anxiety was tested in elevated plus maze. The elevated plus maze consists of two opposite open arms (50 cm long, 10 cm wide) and two opposite closed arms (50 cm long, 10 cm wide, 40 cm high), elevated 50 cm above the floor. The rats were individually placed in the center of the maze with their heads facing the closed arm. During the behavioral test (5 min), the number of entries into the open and closed arms and the time spent on them were measured.

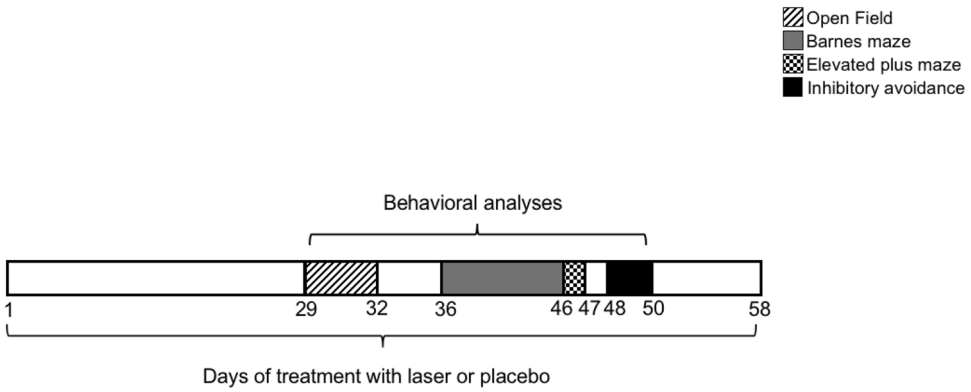


**Fig. 2** Experimental design. Male Wistar rats from the young control (YC; n = 15), young laser (YL; n = 15), aged control (AC; *n* = 16), and aged laser (AL; *n* = 18) groups were exposed to a laser treatment or placebo over 58 consecutive days. From the 29th day of the experiment, the rats were submitted to four different behavior tests in the following sequence: exploratory activity and habituation in open field over three days, four days of rest, spatial learning and memory in the Barnes maze test for ten consecutive days (from the 36th to the 45th days of treatment), anxiety in elevated plus maze (46th day of treatment), one day of rest, aversive memory in inhibitory avoidance (48th and 49th days of treatment), and nine days of behavioral washout (from the 50th to the 58th day of treatment)

#### Inhibitory Avoidance

Aversive memory was tested in inhibitory avoidance. The inhibitory avoidance apparatus consists of an acrylic box (50 × 25 × 25 cm) with parallel stainless-steel bars (1 mm diameter) spaced 1 cm apart in the floor. A platform (7 × 25 cm) was placed against the left wall. For the inhibitory avoidance training, the animals were placed on the platform and allowed to step down onto the grid for up to 60 s. Immediately after stepping down on the grid with all four paws, animals received a 0.6 mA/s scrambled foot shock. The animals stayed in the apparatus for 60 s after the aversive stimulus was given and were immediately returned to their home cages. Ninety minutes (short-term memory) and 24 h (long-term memory) after the training session (aversive stimulus), the animals were again placed in the apparatus and the latency to step down was measured. If the animal did not step off the platform within 5 min, the experiment was terminated. In the long-term memory analysis, no aversive foot stimulus was delivered and the step-down latency was used to evaluate aversive memory.

### Analysis of Chemokine and Cytokine Levels

#### Tissue Preparation

Twenty-four hours after the final laser session (59th day of experiment), the rats were euthanized by decapitation and their cerebral cortex and hippocampus were immediately collected and frozen. The tissue samples were homogenized in ice-cold RIPA lysis buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.5% sodium deoxycholate, 1% NP-40, 0.1% SDS) with freshly added protease (Cat# M222–1 ml; Lot# 1295C056; Amresco) and phosphatase (Cat# B15001-A and B; Lot# 510,011; Biotool) inhibitors. Homogenates were centrifuged at 10,000 × g for 10 min at 4 °C and supernatants were transferred to a new tube.

#### Cytokine and Chemokine Measurements

The levels of cytokines and chemokines in the brain samples were quantified using the Milliplex® MAP rat cytokine/chemokine magnetic bead panel assay (RECYTMAG-65 K, Merck Millipore) following the manufacturer's specifications. This multiplex immunoassay allows the simultaneous quantification of 25 molecules: G-CSF, GM-CSF, eotaxin, IL-1alpha, leptin, MIP-1alpha, IL-4, IL-1beta, IL-2, IL-6, EGF, IL-13, IL-10, IL-12p70, IL-5, IL-17alpha, IL-18, MCP-1, IP-10, VEGF, fractalkine, LIX, MIP-2, TNF-alpha, and RANTES. The plates were read on a Luminex™ Magpix™ instrument and results were analyzed with the Milliplex Analyst 5.1 Software using a Logistic 5P Weighted regression formula to calculate sample concentrations from the standard curves.

### Statistical Analysis

Statistical procedures were conducted using two-way ANOVA or two-way ANOVA with repeated measures. The Z-score was used to remove outlier values (± SEM): one outlier of leptin (AL), IL-5 (YC), IL-18 (AC), and RANTES (AL); two outliers of EGF (YC and YL) and IP-10 (YC and AC) in the cortex; and one outlier of GM-CSF (YL), IL-2 (YL), EGF (YC), IL-12p70 (YC), IL-5 (AL), and fractalkine (YC) in the hippocampus. All analyses were performed using the Statistical Package for the Social Science (SPSS Inc, IBM, version 221.0, Chicago, IL, USA). A statistical difference was considered when the *P*-value was lower than 0.05. All plots were acquired using the GraphPad Prism (6.0).

## Results

### Exploratory Activity in Open Field

Exploratory activity was measured in an open field apparatus on the 29th day of the experiment. Two-way ANOVA showed a significant difference in age [central locomotion ($F_{(1,60)} = 7.309$; $p = 0.009$), peripheral locomotion ($F_{(1,60)} = 14.139$; $p < 0.0001$), and total locomotion ($F_{(1,60)} = 15.543$; $p < 0.0001$)], but no significant effect in group [central locomotion ($F_{(1,60)} = 0.201$; $p = 0.655$), peripheral locomotion ($F_{(1,60)} = 0.007$; $p = 0.934$), and total locomotion ($F_{(1,60)} < 0.0001$; $p = 1.000$)] and interaction (age*group) [central locomotion ($F_{(1,60)} = 1.048$; $p = 0.310$), peripheral locomotion ($F_{(1,60)} = 0.637$; $p = 0.428$), and total locomotion ($F_{(1,60)} = 0.835$; $p = 0.365$)] (Fig. 3). These data suggest that the laser exerts no effect on the locomotion and exploration of young and aged rats in the open field test.

### Habituation in Open Field

Habituation was measured in the open field apparatus over three days (from the 29th to the 31st day of treatment). Statistical analysis of central, peripheral, and total locomotion data was conducted independently by ANOVA with repeated measures. In the central locomotion variable, an effect of day was observed ($F_{(2,120)} = 4.641$; $p = 0.011$), but not group ($F_{(3,60)} = 1.379$; $p = 0.258$) and interaction ($F_{(6,120)} = 1.534$; $p = 0.173$). In the peripheral locomotion variable, an effect of day ($F_{(2,120)} = 4.402$; $p = 0.014$) and group ($F_{(3,60)} = 5.913$; $p = 0.001$) was observed, but not interaction ($F_{(6,120)} = 0.560$; $p = 0.761$). In the total locomotion variable, an effect of

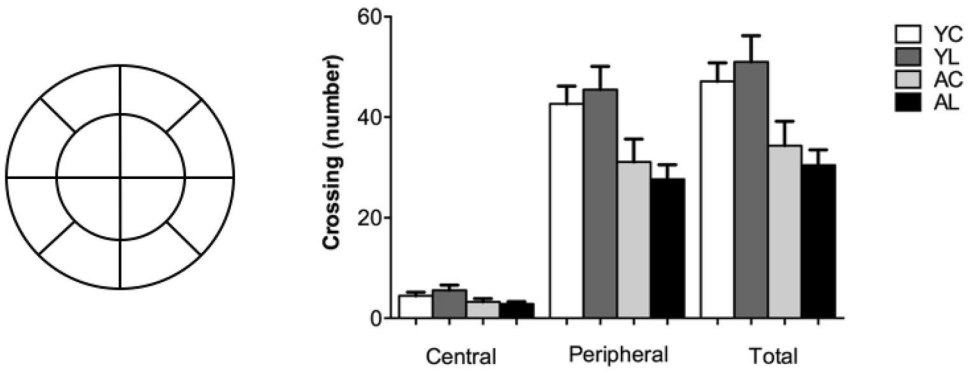


**Fig. 3** Open field quadrants (left) and exploratory activity from young control (YC; $n=15$), young laser (YL; $n=15$), aged control (AC; $n=16$), and aged laser (AL; $n=18$) groups. Exploratory activity is expressed as number of quadrants crossed (locomotion units) for both the four central quadrants (center) and the eight peripheral quadrants (periphery). Total locomotion corresponds to the sum of central and peripheral locomotion. No significant effect was observed (two-way ANOVA)

day ($F_{(2,120)}=5.565$; $p=0.005$) and group ($F_{(3,60)}=5.526$; $p=0.002$) was observed, but not interaction ($F_{(6,120)}=0.615$; $p=0.718$) (Fig. 4). These data suggest that the laser does not alter the habituation (non-associative memory task) of young and aged rats.

## Spatial Learning and Memory in the Barnes Maze

Spatial learning and memory were assessed in the Barnes maze for ten consecutive days (from the 36th to the 45th day of treatment). Two-way ANOVA with repeated measures showed an effect of day ($F_{(9,540)}=72.301$; $p<0.0001$), group ($F_{(3,60)}=2.972$; $p=0.039$), and interaction (group*day) ($F_{(27,540)}=3.473$; $p<0.0001$). When Bonferroni post hoc analysis was performed, it was noted that young groups [YC ($p=0.034$), YL ($p<0.0001$)] and AL group ($p=0.003$) learned to find the true hole earlier (on the 6th day of testing) than the AC group (learning of the task in this group occurred only on the 7th test day; $p=0.003$) (Fig. 5).

In addition, on the 8th day of the test, a better performance in finding the true hole was noted in the YL group in comparison to the YC group ($p=0.002$). This was also found in the aged animals because a lower latency was observed in the AL group when compared to the AC group on the 9th ($p=0.001$) and 10th ($p=0.045$) days of the test. These data show that animals in the PBMT group showed better performance in finding the true hole over the test days than the control group. In addition, the PBMT group was able to rejuvenate the spatial mnemonic damage of the aged rats, resulting in a performance close to that of the rats in the YC group (Fig. 5).

## Anxiety in Elevated Plus Maze

Anxiety was assessed using the elevated plus maze where rats can freely explore closed or open arms on a high platform (46th day of treatment). An increase in time spent in the closed arms is related to anxiety. The statistical

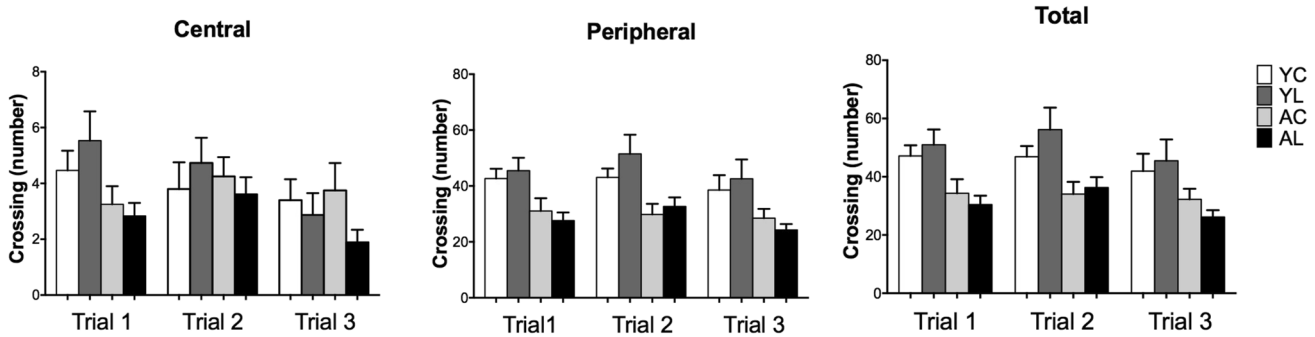


**Fig. 4** Habituation memory from young control (YC; $n=15$), young laser (YL; $n=15$), aged control (AC; $n=16$), and aged laser (AL; $n=18$) groups over three consecutive days. No significant difference in central, peripheral, or total locomotion was found among groups (two-way ANOVA with repeated measures)

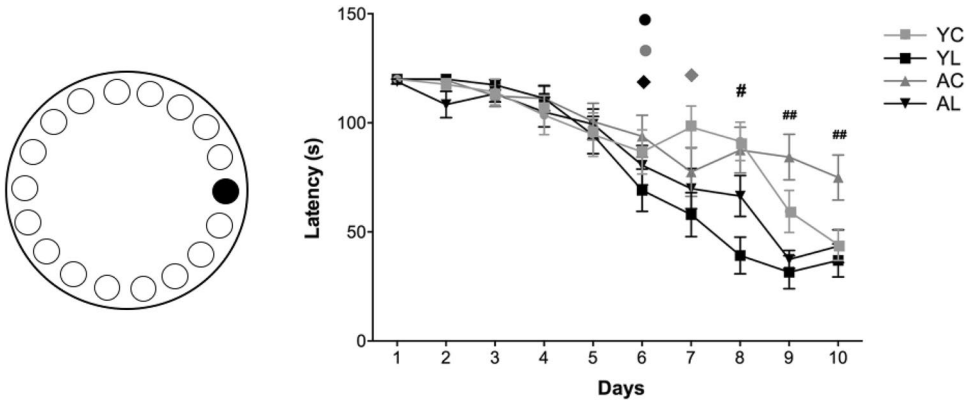


**Fig. 5** A schematic representation of the Barnes maze (left) and latency to find the hole over the ten consecutive days (120 s/day) of a test in the Barnes labyrinth in animals of all groups: young control (YC; $n=15$), young laser (YL; $n=15$), aged control (AC; $n=16$), and aged laser (AL; n = 18). A statistical difference was observed on the sixth test day in relation to the first day in the animals of the YC (□), YL (□) and AL (◆) groups; in the AC group this difference was observed on the seventh test day (◆). In addition, on the eighth day of the test, a significant decrease in latency was noted to find the true hole between the YL and YC groups ($^{\#}$). This same effect was found in aged rats because there was a significant reduction in the mnemonic performance of the animals of the AL group compared to the animals of the AC group on the ninth and tenth days ($^{\#\#}$) ($p<0,05$; two-way ANOVA with repeated measures)

analyses were performed by two-way ANOVA. No significant effect was observed in the variables analyzed: time on closed arms [age ($F_{(1,60)}=2.808$; $p=0.099$), group ($F_{(1,60)}=2.013$; $p=0.161$), and interaction ($F_{(1,60)}=1.350$; $p=0.250$)]; time on open arms [age ($F_{(1,60)}=2.021$; $p=0.160$), group ($F_{(1,60)}=0.023$; $p=0.881$), and interaction ($F_{(1,60)}=0.268$; $p=0.607$)]; entries on closed arms [age ($F_{(1,60)}=0.477$; $p=0.493$), group ($F_{(1,60)}=3.267$; $p=0.076$), and interaction ($F_{(1,60)}=3.460$; $p=0.068$)]; and entries on open arms [age ($F_{(1,60)}=1.749$; $p=0.191$), group ($F_{(1,60)}=0.045$; $p=0.833$), and interaction ($F_{(1,60)}<0.0001$; $p=0.995$)] (Fig. 6). These data suggest that PBMT exerts no anxiolytic effect on the elevated plus maze test in young and aged rats.

## Aversive Memory in Inhibitory Avoidance

Aversive memory was measured in the inhibitory avoidance (48th and 49th days of treatment). The step-down latency was used to evaluate short- (90 min after the training session) and long-term (24 h after the training session) aversive memory. Two-way ANOVA showed no significant difference in age ($F_{(1,57)}=0.737$; $p=0.394$), group ($F_{(1,57)}=0.463$; $p=0.499$), and interaction ($F_{(1,57)}=0.273$; $p=0.604$) when

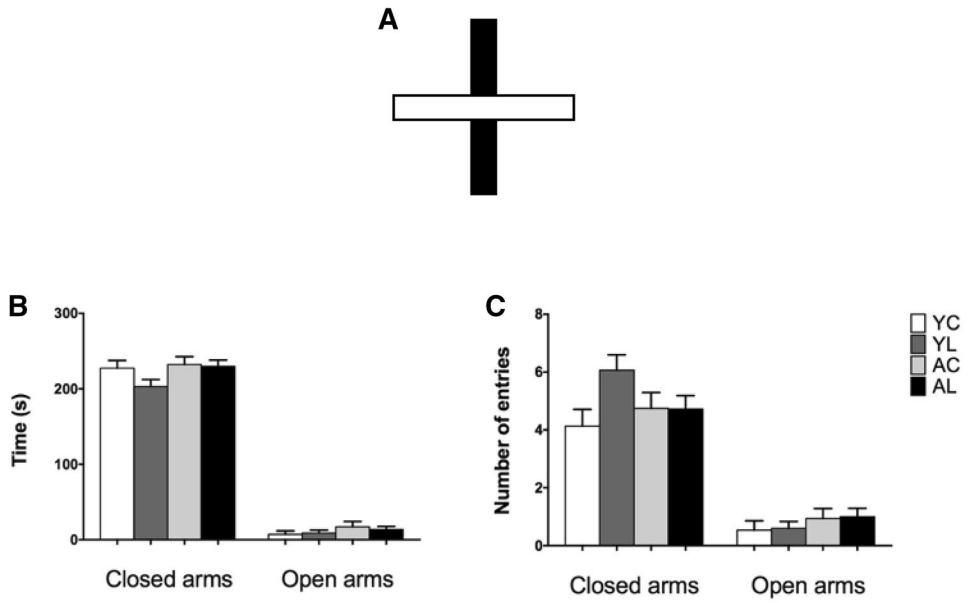


**Fig. 6** **a** Open and closed arms of the elevated plus maze. **b** Time and **c** Entries on closed and open arms by young control (YC; $n=15$), young laser (YL; $n=15$), aged control (AC; $n=16$), and aged laser (AL; $n=18$) groups. No significant difference was observed (two-way ANOVA)

the short-term memory was evaluated (Fig. 7a). When long-term memory was evaluated, a significant difference was found between groups ($F_{(1,57)}=9.856$; $p=0.003$), but not in age ($F_{(1,57)}=2.213$; $p=0.142$) or interaction ($F_{(1,57)}=0.448$; $p=0.506$) (Fig. 7b). These data show that the laser has no effect on the short- and long-term aversive memory.

### Cortical and Hippocampal Levels of Cytokines and Chemokines

The results of the two-way ANOVA are presented in Supplementary Tables S1, S2, S3, and S4. No significant effect was observed in the cortical levels of.

G-CSF ($F_{(1,21)}=0.504$; $p=0.486$), eotaxin ($F_{(1,21)}=3.088$; $p=0.093$), IL-1alpha ($F_{(1,21)}=2.323$; $p=0.127$), leptin ($F_{(1,20)}=1.246$; $p=0.278$), MIP-1alpha ($F_{(1,21)}=0.399$; $p=0.535$), IL-4 ($F_{(1,21)}=1.202$; $p=0.285$), IL-1beta ($F_{(1,21)}=1.267$; $p=0.273$), IL-2 ($F_{(1,21)}=0.031$; $p=0.862$), EGF ($F_{(1,19)}=1.963$; $p=0.177$), IL-13 ($F_{(1,21)}=3.849$; $p=0.063$), IL-17alpha ($F_{(1,21)}=0.015$; $p=0.902$), IL-18 ($F_{(1,20)}=0.180$; $p=0.676$), IP-10 ($F_{(1,19)}=1.402$; $p=0.251$), VEGF ($F_{(1,21)}=0.052$; $p=0.822$), fractalkine ($F_{(1,21)}=0.884$; p = 0.358), MIP-2 ($F_{(1,21)}=2.308$; $p=0.144$), and RANTES ($F_{(1,20)}=0.965$; $p=0.338$). In the other cytokines/chemokines, we noted significant effect ($p<0.05$). When Bonferroni post hoc analysis was performed, we noted that laser treatment reduced the cortical levels of GM-CSF ($p<0.0001$), IL-10 ($p=0.003$), MCP-1 ($p=0.012$), LIX ($p=0.006$), and TNF ($p<0.0001$) in young rats. Also, we noted a decrease in the cortical levels of GM-CSF ($p<0.0001$), IL-6 ($p=0.005$), MCP-1 ($p=0.028$), LIX ($p<0.0001$), and TNF ($p<0.0001$) in the animals of the AC group compared to the animals of the YC group. In addition, the laser increased IL-6 ($p=0.046$), IL-10 (p = 0.008), and TNF-alpha ($p=0.027$) levels and reduced IL-5 cortical levels in aged rats ($p=0.005$) (Fig. 8). Despite finding a significant difference in the two-way ANOVA in the cortical levels of IL-12p70, no effect was observed in the Bonferroni post hoc analysis.

In the hippocampus, no effect was noted in the G-CSF ($F_{(1,20)}<0.0001$; $p=0.988$), GM-CSF ($F_{(1,19)}=3.447$; $p=0.079$), eotaxin ($F_{(1,20)}=0.002$; $p=0.964$), IL-1alpha ($F_{(1,20)}=3.194$; $p=0.089$), leptin ($F_{(1,20)}=0.018$; $p=0.896$), MIP-1alpha ($F_{(1,20)}=2.125$; $p=0.160$), IL-4 ($F_{(1,20)}=0.191$; $p=0.667$), IL-1beta ($F_{(1,20)}=0.203$; $p=0.657$), IL-2 ($F_{(1,19)}=1.074$; $p=0.313$), IL-6 ($F_{(1,20)}=0.202$; $p=0.658$), EGF ($F_{(1,19)}=2.319$; $p=0.144$), IL-13 ($F_{(1,20)}=0.080$; $p=0.780$), IL-10 ($F_{(1,20)}=1.003$; $p=0.329$), IL-12p70 ($F_{(1,19)}=0.270$; $p=0.610$), IL-5 ($F_{(1,19)}=0.069$; $p=0.795$), IL-17alpha ($F_{(1,20)}=0.160$; $p=0.694$), IL-18 ($F_{(1,20)}=1.472$; $p=0.239$), MCP-1 ($F_{(1,20)}=0.472$; $p=0.500$), VEGF ($F_{(1,20)}=3.878$; $p=0.063$), LIX ($F_{(1,20)}=0.141$; $p=0.711$), MIP-2 ($F_{(1,20)}=1.639$; $p=0.215$), TNF-alpha ($F_{(1,20)}=0.430$; $p=0.520$), and RANTES ($F_{(1,20)}=4.189$; $p=0.054$) levels. However, was observed a significant effect in the IP-10 ($F_{(1,20)}=4.892$; $p=0.039$) and fractalkine ($F_{(1,19)}=5.819$; $p=0.026$). When Bonferroni post hoc analysis was performed, an increase in the IP-10 levels was observed in control aged rats when compared to control young rats ($p=0.025$). Interestingly, the laser was able to reduce the exacerbated levels of IP-10 ($p=0.038$) back to levels seen in young animals. In addition, PBMT reduced the level of fractalkine ($p=0.033$) from aged rats (Fig. 9). Taken together, these data show that PBMT changes the levels of neuroinflammatory markers in young and aged rats (Table 1).

## Discussion

The purpose of our study was to investigate the cognitive performance and the levels of pro- and anti-inflammatory cytokines and chemokines in the brain of young (4 months) and aged (20 months) rats exposed to a chronic treatment with a diode laser of 810 nm wavelength and 100 mW power. Our results indicate that PBMT was able to improve learning and spatial memory and modulate the neuroinflammatory profile of young and aged rats.

### Photobiomodulation and Behavioral Tests

In the present study, habituation memory in the open field, anxiety in the elevated plus maze test, and short- and long-term aversive memory in the inhibitory avoidance were not significantly altered by PBMT. Our study is the first

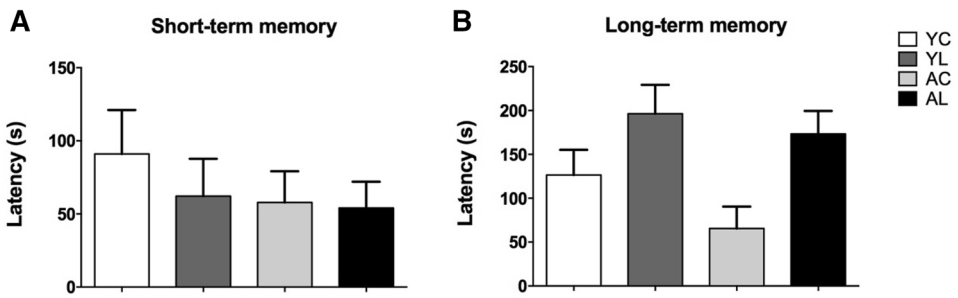


**Fig. 7** Inhibitory avoidance. **a** Short-term memory test and **b** Long-term memory test by young control (YC; $n=15$), young laser (YL; $n=15$), aged control (AC; $n=15$), and aged laser (AL; $n=16$) groups. No significant difference was observed in either one of the analyzed times (two-way ANOVA)

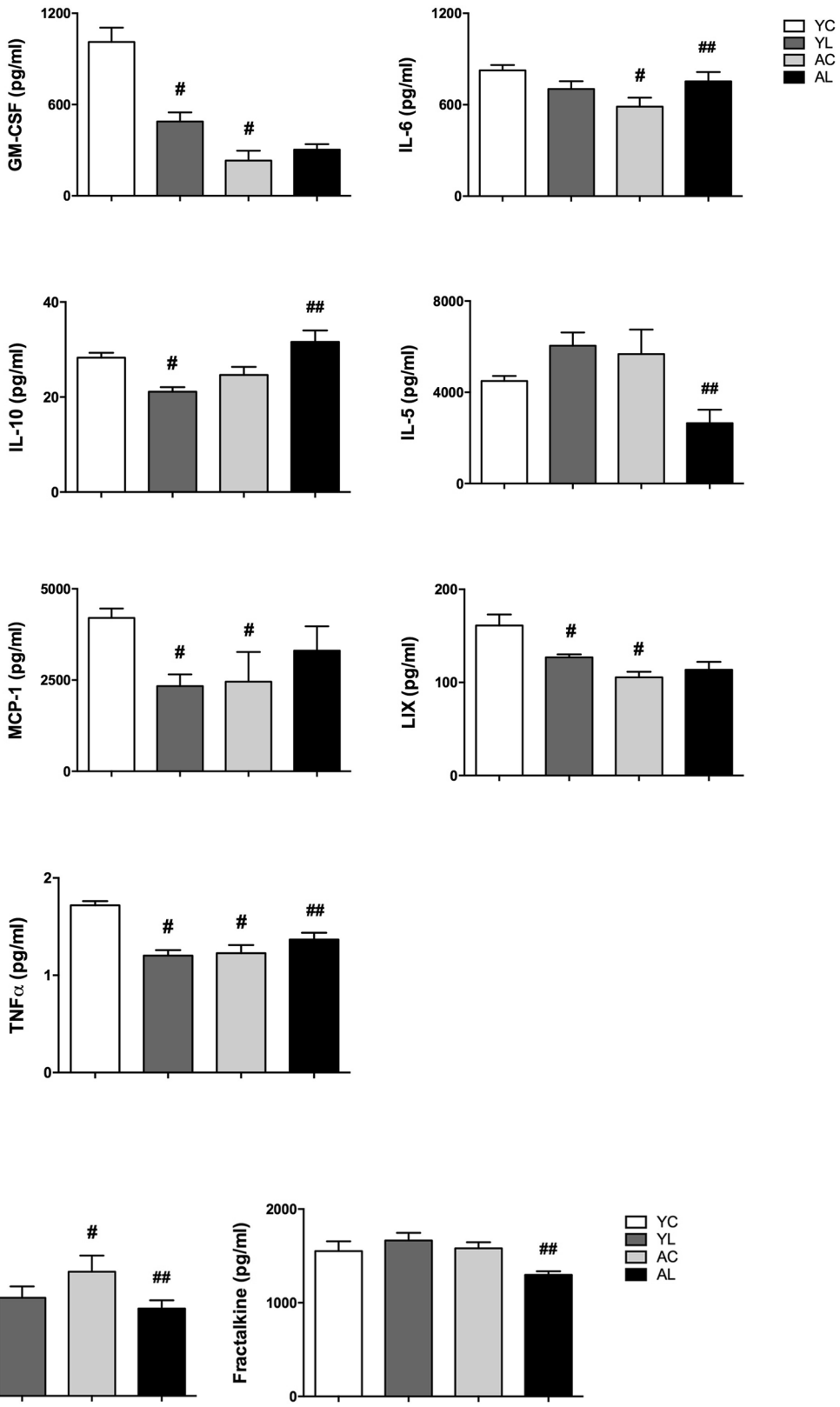


**Fig. 8** Cortical levels of GM-CSF, IL-6, IL-10, IL-5, MCP-1, LIX, and TNFα in rats from young control (YC; $n=7$), young laser (YL; $n=7$), aged control (AC; $n=5$), and aged laser (AL; $n=6$) groups. A significant decrease in GM-CSF, IL-10, MCP-1, LIX, and TNF levels were found in the YL group when compared to the YC group (#). A significant decrease in the levels of GM-CSF, IL-6, MCP-1, LIX, and TNF were noted in the AC group, compared to the YC group (#). In addition, the laser increased IL-6, IL-10, and TNFα levels and reduced IL-5 levels in aged rats (##) ($p<0,05$; two-way ANOVA)

**Fig. 9** Hippocampal levels of IP-10 and Fractalkine in rats from young control (YC; $n=7$), young laser (YL; $n=7$), aged control (AC; $n=5$), and aged laser (AL; $n=5$) groups. A significant increase in IP-10 levels was found in the AC group when compared to the YC group (#). A significant decrease in IP-10 and Fractalkine levels were noted in the AL group compared to the AC group (##) ($p<0,05$; two-way ANOVA)

**Table 1** Laser parameters used in the present study

| Parameter (unit) | Measurement method or value information source |
|---|---|
| Center wavelength (nm) | 810 |
| Operating mode | Continuous wave |
| Average radiant power (W) | 0.1 |
| Aperture diameter (cm) | 0.6 |
| Irradiance at aperture (mW/cm$^2$) | 0.0357 |
| Beam divergence (degree) | 21.71 |
| Beam shape | Circular |
| Beam spot size (cm$^2$) | 0.028 |
| Irradiance at target (mW/cm$^2$) | 0.0357 |
| Exposure duration/point (s) | 30 |
| Radiant exposure (J/cm$^2$) per session | 535.7 |
| Number of points irradiated | 5 |
| Delivery mode | Contact mode |
| Number and frequency of sessions | One session/day for 58 consecutive days |
| Total radiant energy (J) per head | 870 |

to evaluate the habituation memory, anxiety, and aversive memory of aged rats exposed to laser treatment. However, these results are intriguing, since previous studies showed beneficial effects of laser treatment in the cognition of different animal models (Xu et al. 2017; Salehpour et al. 2017, 2019). For example, Xu and colleagues (2017) showed that an animal model of anxiety (*Ahi1* knockout mice) exhibited fewer anxiety symptoms in the forced swimming test and tail suspension 7, 14, and 21 days after laser treatment. Based on the data described in the literature, we expected that the laser therapy protocol used in our study could improve behavioral performance of young and aged rats on open field, elevated plus maze, and step-down inhibitory avoidance apparatus. We found no effect of PBMT on these tests. We only noted PBMT-induced benefits in the Barnes maze test. Young and aged rats submitted to transcranial laser showed better performance in finding the true hole over the days of the test than control rats. This laser benefit on rat's spatial memory in the Barnes maze was also observed in a previous study conducted in an animal model of aging (D-galactose-induced aging (Salehpour et al. 2017) and of transient global brain ischemia (Salehpour et al. 2019).

## Photobiomodulation and Inflammatory Response in Young Rats

The laser treatment altered the cortical and hippocampal expression of inflammatory markers. The laser reduced the cortical levels of GM-CSF, MCP1, LIX, and TNF-alpha in young rats. These results are optimistic, since GM-CSF is related to several infectious and inflammatory diseases (Shim et al. 2012). For example, Shang et al. (2016) noted that high production of GM-CSF in the brain parenchyma of AD patients promotes monocyte transmigration across the blood–brain barrier. MCP-1 induces expression and secretion of RANTES chemokine in T cells and MIP-3α in brain ischemia model animals, increasing inflammatory response (Che et al. 2001; Chen et al. 2003; Terao et al 2009). The expression of LIX during brain ischemia, close to its receptor, measures the activation of JNK and p38 signal pathways which are linked to inflammation and cell death (Jeyaseelan et al. 2005; Shin et al. 2014). TNFα is a pro-inflammatory cytokine involved in innate immune response (Clark 2007) that encodes inflammatory enzymes, such as inducible nitric oxide (iNOS) and cyclooxygenase-2 (COX-2), related to various neurodegenerative diseases, such as AD (Allan and Rothwell 2003; Heneka 2006).

## Photobiomodulation and Inflammatory Response in Aged Rats

Our data show that laser treatment alters the inflammatory response of aged rats, corroborating with studies that show these effects on the retina (Kokkinopoulos et al. 2013; Sivapathasuntharam et al. 2017) and brain (El Massri et al 2018) of aged animals. In our study, the laser increased the cortical expression of IL-6, TNF-alpha, and IL-10 in aged rats. IL-6 has pro and anti-inflammatory properties (Scheller et al 2011; Ataie-Kachoie et al. 2014; Yao et al. 2014) For example, this cytokine is involved in the activation of the immune system, but also in the regulation of metabolism and in many neural functions (Scheller et al. 2011). These effects depend on the activation mechanisms and length of exposure to the cytokine (Ataie-Kachoie et al. 2014). One hypothesis for this may be the anti-inflammatory effect of PBMT, since the reduced IL-6 cortical levels in aged rats were restored by laser treatment. TNF-alpha is up-regulated in many degenerative diseases. However, the TNF-alpha can exert a neuroprotection mediated by TNFR2 activation against glutamate-induced excitotoxicity (Marchetti et al. 2004). This cytokine induces persistent NF-kB activation involving PI3K and Akt, which is strongly enhanced by N-methyl-D-aspartate receptor activation, in turn is essential for neuronal survival and synaptic plasticity (Marchetti et al. 2004; Mattson 2005). Furthermore, these data are consistent with a study conducted by our research group. We noted that laser treatment reduced the cortical metabolic pathway of glutamate in aged rats (Cardoso et al. 2021). IL-10 is an anti-inflammatory cytokine that inhibits the activation of pro-inflammatory proteins (Ye and Johnson 2001). Studies show that IL-10 blockage triggers neuronal damage and behavioral impairment (Grilli et al. 2000; Krzyszton et al. 2008). These data

suggest that during aging, reduced levels of IL-10 may result in vulnerability and neuronal dysfunction.

Supporting the positive effect of PBMT on the aged brain, the laser therapy reduced the cortical expression of IL-5 in aged rats. IL-5 is a pro-inflammatory cytokine that induces proliferation of microglia and increases IL-9 levels under excitotoxic conditions (Liva and Vellis 2001). In this sense, the aged brain is characterized by microglia reactivity and high levels of pro-inflammatory cytokines (Jurgens and Johnson 2012). Moreover, these findings corroborate studies of gene expression profiles of human brains and aged animals. It is observed that the expression of genes involved in oxidative stress, inflammation, and glial activation increases during the aging process, while genes linked to synaptic function and growth factors decrease throughout life (Bishop et al. 2010; Blalock et al. 2003; Godbout et al. 2005; Lee et al. 2000).

In the hippocampus, we found an increase in IP-10 expression in rats from the AC group compared to rats from the YC group. Interestingly, the laser therapy reduced exacerbated levels of this chemokine in aged rats. In addition, PBMT also reduced fractalkine levels in aged rats. These results are interesting since studies have shown that these chemokines are elevated in the brains of animals with AD (Hanzel et al. 2014; Scholtzova et al. 2014). Moreover, it is noted that Aβ plaques can stimulate the production of IP-10 and fractalkine, along with memory loss (Lai et al. 2013; Wu et al. 2013). It is observed that increased expression of chemokines and cytokines induces neuritic dystrophy, triggering increased expression and phosphorylation of neurofilaments and tau protein (Sheng et al. 2000). In addition, chemokines can interact with each other. It is known that the interaction between SDF-1α and CXCR4 in astroglioma cells induces the expression of MCP-1, IL-8, and IP-10 through the ERK signaling pathway, which is linked to inflammation and angiogenesis (Oh et al. 2001).

### Impact of Photobiomodulation on Young and Old Brain

Our data show that the greatest effects of PBMT on behavioral performance and neuroinflammatory response were in aged rats, corroborating with the findings of Shinhmar and collaborators (2020). They noted that laser treatment improved the rod and cone function in elderly people, but not in younger individuals, possibly, because age-related mitochondrial decline has not yet impacted young individuals (Shinhmar et al. 2020).

### Limitations

Although our data are promising, it is worth noting that the study has limitations. We cannot affirm that our behavioral and inflammatory results are closely related. In other words, we cannot assume that the behavioral performance of the animals is linked to changes in brain inflammatory levels since our analyses occurred on different days. The behavioral analyses were performed from the 29th to the 49th days of treatment, while biochemical analyses were performed on the 58th day of treatment.

## Conclusion

Despite that, we consider transcranial photobiomodulation to be a non-pharmacological therapeutic tool with potential for age-related brain disorders, mainly for improving memory and restoring inflammatory levels.

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## Declarations

**Conflict of interest** The authors declare that they have no conflicts of interest. All the authors read and approved the final manuscript.

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