Chapter

Transcranial Red LED Therapy: A Promising Non-Invasive Treatment to Prevent Age-Related Hippocampal Memory Impairment

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Abstract

The hippocampus is an integral portion of the limbic system and executes a critical role in spatial and recognition learning, memory encoding, and memory consolidation. Hippocampal aging showed neurobiological alterations, including increased oxidative stress, altered intracellular signaling pathways, synaptic impairment, and organelle deterioration such as mitochondrial dysfunction. These alterations lead to hippocampal cognitive decline during aging. Therefore, the search for new non-invasive therapies focused on preserving or attenuating age-related hippocampal memory impairment could have of great impact on aging, considering the increasing life expectancy in the world. Red light Transcranial LED therapy (RL-TCLT) is a promising but little explored strategy, which involves red light LED irradiation without surgical procedures, safe and at a low cost. Nevertheless, the precise mechanism involved and its real impact on age-related cognitive impairment is unclear, due to differences in protocol, wavelength applied, and time. Therefore, in this chapter, we will discuss the evidence about RL-TCLT and its effects on the hippocampal structure and function, and how this therapy could be used as a promising treatment for memory loss during aging and in age-related diseases such as Alzheimer's Disease (AD). Finally, we will mention our advances in Red 630-light-Transcranial LED therapy on the hippocampus in aging and AD.

Keywords: aging, hippocampus, memory, LED therapy, mitochondria

1. Introduction

Aging is a biological process characterized by a general decline in cell function. Life expectancy is increasing and has turned aging into a social problem in the world. The brain is one of the organs that is most affected by age [1, 2], therefore new investigations into safe and non-invasive treatments to reduce age-related brain damage and subsequent cognitive impairment are of critical importance. The aging brain displays synaptic alterations that negatively affect cognitive capacity, especially memory. The hippocampus mediates the formation of new memories and age-related hippocampal dysfunction compromises learning and memory processes [3].

Interestingly, in hippocampal memory loss, mitochondrial dysfunction plays a central role. Synaptic and mitochondrial dysfunction are early events in aging, mutually influenced, triggering age-associated memory defects [4]. Then, the need arises to find new strategies that can help elderly people to pass a better old age, without forgetting their memories or their history.

A promising but little explored strategy is the application of non-invasive cell stimulation with specific light types. Photobiomodulation is the use of light to stimulate or regenerate organs and tissues. Red-near-infrared (800-1100 nm) and red (600 nm) wavelengths of light-emitting diodes (LED) have been used for a range of therapeutic purposes [5–7]. These wavelengths could penetrate through the skin and have the potential to improve the cellular function of compromised tissue [5, 7]. Red-near-infrared and red LED therapy involves the interaction of photons with molecules in the cells [5, 8, 9]. Specifically, Transcranial LED therapy (TCLT) defines the limited application of LED therapy to the brain. The LED light travels through the layers of the scalp and skull to reach brain cells [10–12]. The brain is commonly irradiated with red (RL) or near-infrared (NIR) light (600-1100 nm), with a total output power of 1-10,000 mW, a power density that has no thermal effects [9]. Several studies have reported the use of brain irradiation with red or near-infrared (600-1100 nm) LED improving tissue repair, blood flow, cicatrization, and recovery following trauma [12–14]; however, the results are variable due to differences in protocols and wavelengths, LED potential, stimulation time the tissue target, the animal model used, as well as the doses or treatment period [13, 15–17].

Diverse experimental and clinical studies have been performed to test transcranial LED therapy with promising results in brain function [9, 14, 16]. Thus, *in vivo* studies using 660 nm and 810 nm Red-light Transcranial LED therapy (RL-TCLT) in a mice model of aging induced by D-galactose in BALB/c mice improved spatial memory and increased mitochondrial function [18]. In transgenic AD mice, RL-LED treatment of the whole body recovered interstitial fluid flow, reduced Aβ deposition in the brain, and alleviate cognitive deficits [19]. Furthermore, studies in patients victims of severe traumatic brain injury (TBI) showed positive effects after RL-TCLT, enhancing their quality of life, by improving their memory, and decreasing affections such as pain, depression, nervousness, and insomnia (**Figure 1**) [12, 20, 21].

Also, complementary *in vitro* studies with 600–850 nm LED irradiation showed light absorption by the cytochrome c oxidase (COX) enzyme, the complex IV of the oxidative phosphorylation (OXPHOS) system located in the electron transport chain (ETC) from the mitochondria [5], leading to the upregulation of the mitochondrial respiratory capacity and increased ATP production [4, 22]. *In vitro* assays also propose that mitochondrial COX act as a photoreceptor that mediates the beneficial effects of photobiomodulation [23]. Nevertheless, until is unclear how COX mediates the beneficial effect regulating energy production, and for this reason, most of the reports concluded that the mechanism underlying the neuroprotective actions of RL-TCLT is not completely understood. More studies are required to determine the biological events that lead to neuroprotection or neuronal repair.

In this chapter, we will summarize the evidence about the studies using Red Light Transcranial LED therapy (RL-TCLT), mainly focused on their positive effect in the brain, and particularly in the hippocampal structure and function. In addition, we will discuss the possible mechanisms involved in the beneficial effects of RL-TCLT, putting particular emphasis on the mitochondria. Finally, we will briefly comment on our main finding using RL-TCLT, as a potential anti-aging therapy.

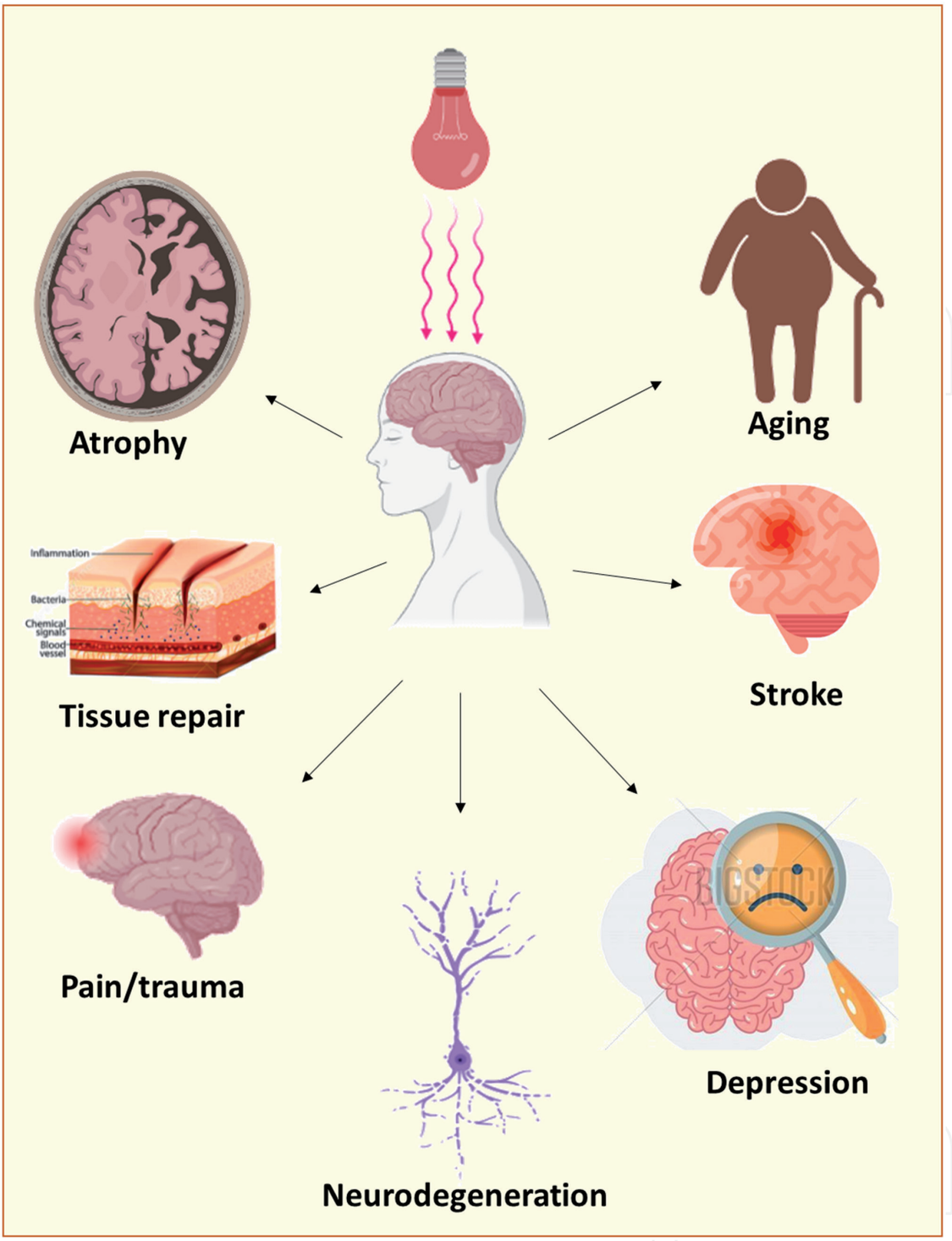

Figure 1.
Beneficial effects of red and near-infrared light on the brain. Diverse reports have shown that irradiation of the brain with red and near-infrared light improves different conditions, including cerebral aging and age-related memory loss, stroke, depression, neurodegeneration in several neurodegenerative diseases, pain and trauma, tissue repair and cicatrization, and atrophy among others.

2. Red light transcranial LED therapy (RL-TCLT): types, devices, uses, and effects

The use of transcranial photobiomodulation is promising in therapeutic and medical benefits for health, with increasing application and projection also in aging and neurodegenerative diseases [9, 24, 25]. The light presents different characteristics that could be used advantageously in the field of health, principally by recent

lighting technologies based on an extensive range of diverse light sources, that have been used for photobiomodulation [8]. Light for therapeutic purposes corresponds to a small fraction of the spectrum of luminous radiation, generally in the visible spectrum [8, 25], where it has a biological effect based on the premise of mammalian cellular metabolism from photoreceptors and chromophores molecules [25].

Diverse devices have been used, including Light Amplification by Stimulated Emission of Radiation (Laser) devices, Low-level light laser therapy, and light-emitting diodes (LED) devices [26]. LED devices are semiconductors that present a high efficiency of electrical energy conversion into optical energy, dissipating little thermal energy [26]. Furthermore, these devices can have widely fluctuating power levels depending on the size, number, and power of the individual diodes [16, 27, 28]. LED devices have been compared with lasers; however, devices irradiating LED are bandwidth (approx 40 nm), beam divergence, incoherent radiation emission, and high optical output power; favoring the absorption of energy by different molecular structures [5, 8]. In addition, LED devices have been considered as a safety by the US Food and Drug Administration (FDA) [29].

Red-Light Transcranial Led Therapy (RL-TLTC) involves power-efficient, low heat-producing light sources that have the potential to deliver high-intensity RL of 600-690 wavelengths, that can be pulsed or continuous [30]. In this therapy, the light goes through the layers of the skin and skull, to stimulate the brain and specific cerebral regions, causing biological responses that result in benefits for the individual [7, 8, 31]. In particular, RL-LED mediated a vibrational absorption process, which produces a photochemical effect that leads to the absorption of photons by specific molecules in the cell [5]. In addition, the wavelength (nm), energy density (J/cm^2), and power density (mW/cm^2) are parameters that determine the effectiveness of RL-TLTC. The wavelength of light used is critical since not all ranges of light used have a similar effect, some ranges present reduced effects such as wavelength in the 700–750 nm range. In contrast, RL-LED at 600–690 nm or 760–900 nm has more impact on the biological tissues [5, 8]. Considering that these parameters of light radiation interact with biological tissue, they cause optical phenomena of reflection, transmission, propagation, and absorption. These characteristics also can present variations depending on tissue irradiated, for example by different concentrations of photoreceptor and chromophores molecules that contain biological tissues, like water, cytochromes, and organic molecules as flavins, hemoglobin, and melanin, among others [5]. When light is absorbed, the photon energy reaches the target molecules producing vibrational, rotational, or electronic processes, which generate diverse effects including photochemical, photo-thermal, photo-mechanical, or photo-electrical stimulation [5].

Interestingly, in the use of RL-TLCT, no standard protocol has been established in the literature; moreover, a few reports have shown studies using diverse parameters as varied wavelength ranges, time (sec/min), irradiance, or power density, and energy density with similar results, and important benefits in the brain health [5]. For example, studies applying Transcranial LED therapy bilaterally with wavelengths of 633 and 870 nm, have shown significant progress in both animals models with acute traumatic brain injury, and patients with acute stroke. In both cases also have been observed an improvement in the cognitive capacity post-treatment with this therapy [20]. Other studies using Low-level Laser Therapy (LLLT), with parameters of energy of 3 J/cm^2, a wavelength of 810 nm, and power density of 20 mW/ cm^2, in primary cultured cortical neurons exposed to oxidative stress reveal that LLLT increased the mitochondrial membrane potential and reduced high ROS levels, reducing neuronal death [32]. Similarly, other studies showed that LLLT has a positive impact on neuronal function in both *in vitro* and *in vivo,* enhancing the metabolic capacity of neurons and cognitive functions including memory [14].

Thus, while the transcranial research using RL LED or laser remains in the initial stages, growing evidence showed that although the RL and NIR-light therapy presents a wide range of characters, can modulate cell activity, including energy metabolism and cell function [25]. This is relevant since these therapies can lead to the improvement of pathological conditions and be in future significant clinical contribution, for example performing clinical treatments that allow helping older persons to prevent or mitigate the age-related cognitive impairment (**Figure 1**).

3. Effects of RL-TCLT on hippocampal structure and function

The effects of RL-TCLT on the nervous system, neuronal repair, and improving cognition are growing, and have been well-documented in cellular, animal models, and human studies [8]. Since the past decade, the use of RL-TCLT as an advanced and non-invasive therapeutic method in several brain-related conditions has attracted interest from researchers in biomedical science, including those conditions or pathologies that manifest memory loss. Nevertheless, the underlying neural mechanisms are not well understood. The hippocampus is a brain structure of special importance in studying aging and cognitive decline, its main function is learning and memory [33]. It is a dorsoventrally elongated area, composed of the dentate gyrus (DG), the cornu ammonis (CA) fields CA1, CA2, and CA3, and the subiculum cortex [34]. The trisynaptic circuit is the main excitatory hippocampal synaptic pathway, formed by 3 neuronal groups: granule cells in the DG, and pyramidal neurons of the CA1 and CA3 [35]. This circuit receives inputs from the superficial layers of the entorhinal cortex via the perforant path to the DG. The DG projects to the CA3, which in turn projects to the CA1. Thus, CA1 projects to the deep layers of the entorhinal cortex, closing the circuit [33, 35, 36]. The hippocampus mediates recognition and spatial memory, by a highly regulated circuit with a high-energy demand [37]. Besides, is important to highlight that the hippocampus is highly susceptible to factors such as mitochondrial dysfunction, stress, inflammation, or physiological process such as aging, accumulating damage that gradually lead to a loss of hippocampal function [33].

Spatial memory gradually decreases with the age, since the hippocampus is critical for this type of memory, and the impairment of hippocampal neurons unequivocally results in spatial memory diminishing [37]. Studies from our and other groups have shown the reduced capacity of aged mice to learn and remember spatial tasks [4, 38, 39]. This is indicated by increased time to find a hidden platform in the Morris Water Maze (MWM) or a hidden chamber in the Barnes Maze (BM), two classic probes to evaluate hippocampus-dependent spatial memory [4, 22]. A report using the senescence-accelerated prone 8 (SAMP8) mice, a mouse model widely used to study oxidative impairment, and age-related brain damage, showed that RL-TCLT at 630 nm for two consecutive months prevents spatial memory loss in 5 month-old (mo) SAMP8 mice, and more importantly rescued the cognitive deficits in SAMP8 mice of 7 mo [40]. This last was accompanied by reduced ROS levels in the brain and increased activity of antioxidant enzymes such as catalase and formaldehyde dehydrogenase [40]. Similarly, TCLT with NIR laser at 810 nm applied in mice exposed to acute sleep deprivation showed reduced hippocampal oxidative damage, increasing the activity of antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx) [16]. Additionally, several studies with cells, animals and in clinical trial conclude that RL-TCLT may have a potential effect on the brain since that has been observed that RL-TCTL protect nerve cells from a future impairment, reducing permanent neuronal damage and increasing their survival. For example, the treatment of K369I tau (K3) mice, a transgenic

mouse model of tauopathies and Alzheimer's Disease, with NIR (600–1000 nm) 20 times four weeks reveal a reduction in the size and number of amyloid-β plaques in the neocortex and hippocampus [41].

Besides, RL-TCLT may have a potential effect promoting both synaptogenesis and neurogenesis [42]. Both processes are essential to facilitating connectivity, neural regeneration, and generate structural changes that help to maintain existing neurons, and to encourage the growth of new neurons and synapses process [43, 44]. In this context, IR-light at 808 nm (350 mW/cm2 and 294 J) was applied in the scalp of a photothrombotic model of ischemic stroke in rats for seven days during 2-minute daily. The authors observed that IR-light therapy significantly attenuated behavioral deficits and infarct volume in cortical regions induced by photothrombotic stroke. This improvement was accompanied by neurogenesis and synaptogenesis, as is indicated by increased immunoreactivity of the proliferative and differentiation markers BrdU, Ki67, DCX, MAP2, spinophilin, and the synaptic marker synaptophysin [45]. Also, other clinical studies reveal positive effects of transcranial LED therapy on cerebral blood fluid (CBF) in patients in a vegetative state or with major depression and anxiety. LED treatment by 20 or 30 min per session, thrice per week over 6 weeks, or two times daily for over seventy days, with different wavelengths of 610, 627, and 810 nm increase CBF, improving cerebral vascular perfusion and reducing brain disorders [9, 46, 47]. Similarly, the application of TC-LLL therapy at 810-nm in mice model of cortical impact and traumatic brain injury reveal increased proliferating neural cells around the lesion, possibly activating regenerative mechanisms such as inducing neurogenesis in the dentate gyrus of the hippocampus [48]. Besides, they observed that the mice treated improved learning and memory reducing cognitive impairment [48, 49].

Thus, while its positive effects have been demonstrated countless times in animal models, they have yet to be proven in broad-scope clinical testing. However, the research that does exist is very promising, strongly indicating that RL-TCLT could be a viable treatment for a broad range of neurological diseases including stroke, traumatic brain injury, Parkinson's disease, Alzheimer's disease, and depression, in addition to providing cognitive enhancement for healthy subjects of advanced age that manifest cognitive impairment.

4. Effects of RL-TCLT on synaptic neurotransmission and synaptic plasticity

Considering that irradiation with RL between 600 and 1200 nm produces changes at molecular, cellular, and tissue levels [50, 51] improving cognitive capacities [52], this enhancement in brain function will result in synaptic neurotransmission and synaptic plasticity potentiation after light treatment [53, 54]. Neurotransmission and synaptic plasticity represent the capacity of synaptic connections to adapt structurally and functionally in a stimulus-dependent manner [43]. Both synaptic neurotransmission and synaptic plasticity can be affected by different factors, such as mitochondrial dysfunction and increased oxidative stress, as well as physiological events including aging, stroke, brain injuries, or neurodegenerative disease, among others [22, 55, 56]. Therefore, treatments focused in maintain or promote neurotransmission and synaptic plasticity are attracting increasing attention. In this context, despite the beneficial effects showed for RL-TCLT on cognition, practically not exist electrophysiological studies using this therapy. As an approximation, we will discuss the studies using transcranial low-level laser light (TC-LLL).

Studies both *in vitro* and *in vivo* have shown that TC-LLL therapy supports neural function, this has been observed principally in reports using transgenic mouse models of Alzheimer's disease (AD) [57]. Meng et al. observed that TC-LLL therapy at 632.8 nm in primary hippocampal neurons treated with full-length $A\beta_{1-42}$ peptide reduced Aβ-induced neurotoxicity. In addition, TC-LLL therapy shows neuroprotective effects decreasing Aβ-induced dendrite atrophy [57]. Also, TC-LLL treatment increased the expression of brain-derived neurotrophic factor (BDNF) in cell line and cultured neurons derived from APP/PS1 transgenic mice, suggesting that this neurotrophin will be modulating dendritic structure, promoting the survival of neurons and dendrite growth, and potentiating synaptic transmission in the CNS [57, 58]. All these results can be explained by the activation of the ERK/CREB/BDNF pathway mediated by TC-LLL therapy [45] because this pathway is involved in the dendritic development of neurons [45, 57, 59]. Therefore, the TC-LLL therapy can induce activation of signaling transduction pathways, and gene transcription, which increases protein expression of different synaptic effectors and modulators, effects that also are potential therapeutic in treating neurodegenerative disease.

Interestingly, the NIR-LED light treatment at 670 nm in the Tg2576 mice model of AD, which progressively accumulated Aβ in their brain [60], indicate that NIR-LED therapy decreased the levels of $A\beta_{1-42}$ at the synapses and Aβ oligomer-induced reduction in long-term potentiation (LTP), relevant processes of neuroplasticity that correlates with memory formation [61]. Therefore, NIR-LED light therapy recovered crucial processes related to synaptic function, necessary to the preservation of cognition abilities [62]. Additionally, studies with photobiomodulation transcranial therapy with wavelengths of 635 nm in a mouse model of depression showed that this treatment reduces glutamate levels and neurotoxicity, improving the depressant behavior. These beneficial effects can be explained by the activation of the PKA pathway and the increased levels of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. In addition, reduce the expression of GluA1, decreasing the glutamatergic neurotransmission. Thus this therapy could rescue excitatory synaptic transmission, improve synaptic plasticity, and have also a potential anti-depressive effect [63].

Thus, several pieces of evidence suggest that the application of transcranial therapy light could be used to improve cellular components associated with the synaptic function [42], which is essential in the maintenance and preservation of cognition, including learning and memory [43]. In addition to the therapeutic effects at the molecular level, it is proposed the generation of changes at the behavioral level, such as cognitive improvement, antidepressant effects, and sleep improvement [42]. Furthermore, this therapy can stimulate neuronal organization or reorganization, therefore it could be extremely promising as a method of stabilization and/or improvement of various brain disorders or nervous system, and neurodegenerative diseases [30, 42]. However, more extensive studies are necessary to evidence all the cellular and molecular mechanisms involved in these encouraging results. This last especially considering that the evidence summarized here consider severe differences in the device and light type used, the protocol of administration, and the study model. Is imperative to advance understanding the multiple targets of red light in the synaptic structure and function.

5. RL-TCLT mechanisms: improving mitochondrial function

Considering the multiple reports revised previously, now known that Red Light therapy, including Red and InfraRed LED light and Red Laser light, have favorable effects on brain structure and function, and especially in the hippocampus

improving cognitive functions [9, 25, 42]. This enhances in cognitive capacity could be explained by the activation of neurogenesis and synaptogenesis [9, 42], as well as by the stimulation of processes related to synaptic plasticity such as LTP [62]. However, any of these events reveal a potential mechanism by which Red light treatment results therapeutic to different affections such as aging, neurodegenerative disease, stroke, and depression among others [42].

Interestingly, all pathological conditions mentioned previously involve, almost in part, dysfunction of hippocampal neurons attributable to mitochondrial defects [64, 65]. For example, mitochondrial dysfunction is considered a hallmark of aging and could be considered one of the factors leading to neurodegeneration [4, 22, 66]. Studies in humans and animal models showed that decreased memory correlates with reduced cerebral energetics metabolism and more specifically to mitochondrial bioenergetics deficits [4, 22, 66]. Therefore, the mitochondrial focus of aging and neurodegenerative diseases is of great interest for the development of a potent and ideally non-invasive anti-aging intervention to improve or attenuate cognitive impairment in the elderly.

Notably, enhanced metabolic functioning is one of the most identifiable properties of irradiate neuronal cells with RL or NIR light, resulting in increased intracellular ATP production [8]. Thus, mitochondrial ATP production is one of the most strongly suggested mechanisms of action of RL therapy [5, 8]; for example, studies using RL-TCLT at 660 nm for 15 sec daily for 2 weeks in aged 18 mo mice improved ATP concentration [16]. More specifically, studies in vitro with RL and NRL LED radiations with a wavelength between 600 and 850 nm have shown that the effects of this treatment are principally attributed to photon absorption by complex IV of the mitochondrial respiratory chain [5]. This mitochondrial complex corresponds to the cytochrome c oxidase (COX) enzyme [22] and it seems that RL increases the activity of this enzymatic complex, leading to enhancement of oxygen consumption and ultimately to mitochondrial respiration [5]. COX is a photo acceptor of RL and NIR light, which generates a redox change in the enzyme [5, 8]. In turn, this causes a transient change in mitochondrial membrane potential (mψ) and increases ATP production [5, 16]. Thus, wavelengths corresponding or near to red will be improving the mitochondrial production of ATP, potentiating the synaptic and cognitive function [5, 42]. Nevertheless, is important to highlight that other works report that RL could inhibit the COX enzyme. In particular, NIR wavelengths of 750 nm and 950 nm reduced the activity of the COX complex. This results in decreased mitochondrial respiration and a loss of mitochondrial membrane potential ($\Delta\Psi m$) [67]. Is surprising to note that the attenuation of mitochondrial function and the concomitant production of superoxide radical reduce neuronal death exposed to oxygen–glucose deprivation and in a mice model of ischemia, an effect that is not observed after other NIR wavelengths that activate COX [67]. Altogether, these contradictory results question the real effect of RL on COX responsible for the beneficial effects of this therapy (**Figure 2**).

On the other hand, several reports showed that RL-LED modulates the levels of reactive oxygen species (ROS) [30, 40]. Studies using RL-LED illumination at 630 nm reduces brain H_2O_2 levels in cultured cells and the brain of SAMP8 mice [40]. This could be explained by an increment in the activity of antioxidant enzymes such as catalase or also could be a consequence of increased mitochondrial function with reduced electron leak [5, 16]; more studies are necessary to evaluate these possibilities. Besides, in this study, the authors showed that RL-LED absorption activates transcription factors that regulate long-lasting effects on gene expression [16], therefore this suggests that Red Light therapy could be a more complex mechanism, at a long time, and not only a transient activation of several enzymes. Other results also showed that Red 635 nm irradiation inhibits the expression of COX

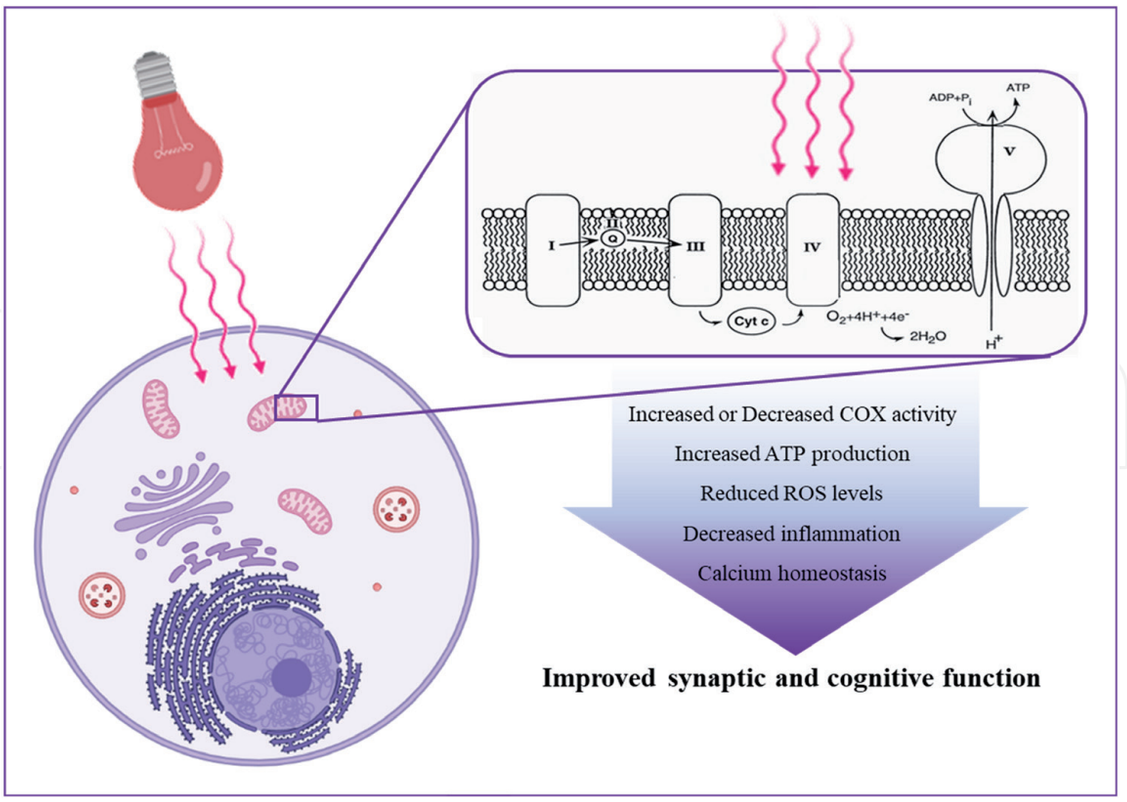

Figure 2.
Mechanism of action proposed to red and near-infrared light and its cellular effects. Transcranial therapy using red and near-infrared light has been proposed to photoactivate the cytochrome c oxidase (COX) enzyme, the complex IV of the electron transporter chain of the mitochondria. However other reports propose that several wavelengths inhibit COX enzyme; modulating ROS and ATP production, calcium homeostasis, and inflammatory processes.

enzyme, reducing ROS levels and mRNA of cytosolic phospholipase A2 (cPLA2) and secretary phospholipase A2 (sPLA2) [68]. This also consequently inhibits the release of PGE2, suggesting an additional anti-inflammatory effect (**Figure 2**).

Additional mechanisms that will be involved in the positive effects of RL-LED implicate Ca^{2+} ions modulation [8, 40]. RL and NIR LED are recognized by water groups formed in the heat/light-gated Ca^{+2} channel. This induces vibrational water energy, which in turn disorganizes the protein structure of the Ca^{+2} channel. This conformational change finally leads to channel opening; modulating intracellular Ca^{+2} levels [5]. This possible mechanism is relevant in neurons, considering that intracellular Ca^{+2} levels are critical to trigger survival or death pathways related to synaptic activity [69].

In summary, despite various mechanisms that could be mentioned such as the potential molecular target of RL and NRL, still is necessary additional research in the field to understand the events that result in synaptic and cognitive function. Possibly these improvements are the result of diverse events occurring simultaneously.

6. Future perspectives of transcranial Red630-light-transcranial LED therapy preventing age-related memory loss: Our advances

Despite diverse studies shown possible molecular targets of RL-LED therapy [5, 8], the precise mechanism underlying the neuroprotective actions of RL-TCLT is not completely understood. Therefore, more studies are required to determine the

biological events that lead to neuroprotection or neuronal repair in both aging and neurodegenerative diseases. Possibly, the main problem related to the incapacity of determining a detailed mechanism is based on the variability of wavelengths, times of treatment, and models used [15, 16, 40, 63, 70]. Therefore, this highlights the need for complete studies using the same mice model, LED dispositive, and therapy protocol, to understand and describe the mechanism(s) underlying the benefits of RL-TCLT.

Interestingly, RL-TCLT at 630 nm in patients with traumatic brain injuries, using a helmet that emits radiation for 30 min, three times per week, for six weeks showed a great reduction in post-traumatic stress symptoms, insomnia, and depression, suggesting improved cognitive function [71]. More importantly, the same RL-TCLT used in aged patients with mild cognitive impairment improves memory in these aged humans. For this reason, and to study the complete effects and mechanisms of RL-TCLT in aging, we designed a unique RL-TCLT device to emit homogeneous light at a wavelength of 630 nm, with 100 J of energy, a power density of 0,35 w/cm^2, and an energy density of 43.5 J/cm^2 in the brain of mice, specifically in the hippocampus.

We applied RL-TCLT to the hippocampus of 7.5mo SAMP8 mice, a mice model of accelerated aging, with an irradiation time of 125 s daily (excluding weekends) for 5 weeks. This protocol is equivalent to the applied to patients with mild cognitive impairment described previously, and the mouse lifespan. We started the RL-TCLT in SAMP8 at 7.5mo because we and other authors showed that the non-transgenic SAMP8 mice present age-related hippocampal memory loss since 6mo and is more evident from 7mo onwards [72]. Interestingly, our results reveal that 7.5mo SAMP8 mice treated with RL-TCLT at the hippocampus improves spatial learning and memory of aged SAMP8 mice. This cognitive improvement will be due to a possible remodeling of the synaptic structure toward more active synapses reducing the risk of excitotoxic events. This is suggested by i) an increase in presynaptic proteins such as synaptophysin (SYP) and Synapsin (SYN) that increase the neurotransmitter release [73], ii) a decrease in the NMDAR subunit NR2B, whose protein levels are related to excitotoxicity [74] and iii) higher Arc protein levels, a marker of synaptic plasticity [75] (Jara et al., manuscript in preparation).

Considering that both memory formation and synaptic activity are highly dependent on energy [76], that mitochondria are the main ATP producer of the cell [22], and that the suggested mechanisms by RL-TCLT target the mitochondria [5], we evaluated different mitochondrial functions in the hippocampus of treated SAMP8 mice with RL-TCLT. Relevantly, we observed increased ATP production, higher activity of the OXPHOS complex II-III, and IV (COX enzyme); suggesting that RL-TCLT directly stimulates mitochondrial bioenergetics function enhancing the activity of other OXPHOS complexes in addition to COX (Jara et al., manuscript in preparation). Similarly, we observed decreased levels of the mitochondrial calcium uniporter (MCU), suggesting that it will result in reduced mitochondrial Ca^{+2} overload and swelling, enhancing mitochondrial Ca^{+2} buffering. In fact, this last was validated in Ca^{+2} overload assays in hippocampal mitochondria from RL-TCLT SAMP8 mice (Jara et al., manuscript in preparation), indicating that RL-TCLT also improves the calcium buffering capacity of the aged hippocampal mitochondria. Whether bioenergetics and calcium buffering enhancing are directly related or are independent mechanisms requires future analysis.

Thus, our results indicated RL-LED-mediated mitochondrial stimulation, which could be transient or permanent. But it is difficult to think that only transient activation of mitochondrial function could explain the improved cognitive effects produced by RL-TCLT treatment. Although mitochondrial ATP production is vital for synaptic communication, it is probably not solely sufficient to

result in improved hippocampal memory. Therefore, is highly probable that other mechanisms are involved in the beneficial effects of RL-TCLT, which result in gene transcription and the consequent cellular remodeling. In concordance with the anterior, we also observed higher PGC-1α protein levels, a transcriptional coactivator considered the main inducer of mitochondrial biogenesis that also regulates mitochondrial function [77]. This suggests that RL-TCLT will stimulate the generation of new mitochondria or the activation of gene-dependent mitochondrial reparation pathways that result in increased mitochondrial function. However, this requires a robust study.

7. Conclusions

In conclusion, despite the majority of treatments using RL-LED therapy are focused on cosmetic applications, RL-TCLT generates cellular effects that will be used to treat different affections including aging and neurodegenerative disease. For this, is necessary to be prudent to decide the more adequate wavelength, light intensity, and duration of therapy, because according to these parameters will found positive or negative effects. In addition, RL-TCLT seems to improve antioxidant defenses and mitochondrial function by enhancing COX IV activity. In concordance with these findings, we also propose that RL-TCLT stimulates mitochondrial function, both enhancing OXPHOS-mediated mitochondrial bioenergetics and calcium buffering capacity. Finally, we suggest that prolonged exposition to RL-TCLT result in a permanent remodeling of the cell, by a mechanism that involves gene transcription, which results in higher synaptic and cognitive function. Future studies are extremely necessary to solve all the questions regarding the benefits of RL-TCLT.

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Conflict of interest

The authors declare no conflict of interest.

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