Biochemical Pharmacology 86 (2013) 447–457

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Neurological and psychological applications of transcranial lasers and LEDs

Julio C. Rojas [a,b], F. Gonzalez-Lima [a,*]

[a] Departments of Psychology, Pharmacology and Toxicology, University of Texas at Austin, Austin, TX 78712, USA
[b] Department of Neurology and Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, TX 75235, USA

ARTICLE INFO

Article history:
Received 8 May 2013
Accepted 15 June 2013
Available online 24 June 2013

Keywords:
Cognitive enhancement
Cytochrome oxidase
Low-level light therapy
Methylene blue
Neuroprotection
Photobiomodulation

ABSTRACT

Transcranial brain stimulation with low-level light/laser therapy (LLLT) is the use of directional low-power and high-fluency monochromatic or quasimonochromatic light from lasers or LEDs in the red-to-near-infrared wavelengths to modulate a neurobiological function or induce a neurotherapeutic effect in a nondestructive and non-thermal manner. The mechanism of action of LLLT is based on photon energy absorption by cytochrome oxidase, the terminal enzyme in the mitochondrial respiratory chain. Cytochrome oxidase has a key role in neuronal physiology, as it serves as an interface between oxidative energy metabolism and cell survival signaling pathways. Cytochrome oxidase is an ideal target for cognitive enhancement, as its expression reflects the changes in metabolic capacity underlying higher-order brain functions. This review provides an update on new findings on the neurotherapeutic applications of LLLT. The photochemical mechanisms supporting its cognitive-enhancing and brain-stimulatory effects in animal models and humans are discussed. LLLT is a potential non-invasive treatment for cognitive impairment and other deficits associated with chronic neurological conditions, such as large vessel and lacunar hypoperfusion or neurodegeneration. Brain photobiomodulation with LLLT is paralleled by pharmacological effects of low-dose USP methylene blue, a non-photic electron donor with the ability to stimulate cytochrome oxidase activity, redox and free radical processes. Both interventions provide neuroprotection and cognitive enhancement by facilitating mitochondrial respiration, with hormetic dose–response effects and brain region activational specificity. This evidence supports enhancement of mitochondrial respiratory function as a generalizable therapeutic principle relevant to highly adaptable systems that are exquisitely sensitive to energy availability such as the nervous system.

1. Introduction

The use of transcranial low-level light/laser therapy (LLLT) to modulate neurological and psychological functions is a paradigm that has gained significant interest among researchers and clinicians in recent years. There is a need for an accurate review that gives proper chronological attribution to the various groups that discovered the transcranial LLLT effects relevant to cognitive enhancement and neuroprotection (listed in Table 1). The fundamental observation that light can be used transcranially to modulate brain function has derived into many significant contributions to forward our understanding of the neurotherapeutic effects of light. Current research focuses on the elucidation of the neurochemical and photobiological mechanisms of action of LLLT and ongoing pre-clinical and clinical investigations aim at determining the role of LLLT in the enhancement of normal brain function, neuroprotection and neural repair. Photobiomodulation with LLLT has become one of the most dynamic and promising fields of experimental neurotherapeutics. Its major appeal is a sound mechanistic theory and the prospective to aid in the treatment of neurological and psychological conditions in a non-invasive, non-expensive and safe manner. Prior reviews have discussed the evidence and potential clinical applications of LLLT in stroke [1] and chronic neurodegenerative conditions [2]. Important aspects of light sources and principles of dosimetry have also been previously summarized. We recently provided an introductory background to photobiology and an overview of the beneficial effects of LLLT on the eye and brain [3]. The objective of the present review is to update on the benefits of transcranial LLLT and the neurochemical mechanisms supporting the cognitive-enhancing and brain-stimulatory effects of transcranial LLLT *via* low-level lasers and light emitting diodes (LEDs) in the red-to-near-infrared

* Corresponding author. Tel.: +1 512 471 5895; fax: +1 512 471 5935.
E-mail address: gonzalezlima@utexas.edu (F. Gonzalez-Lima).

0006-2952/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bcp.2013.06.012

Table 1
Transcranial low-level light/laser therapy studies relevant to neuroprotection and cognitive enhancement.

Date	Reference	Relevance	Source	Parameters	Effects
2004	Lapchak et al. [22]	Embolic stroke	Laser	808 nm, 25 mW/cm^2, 15,000 J/cm^2, continuous	Improved motor function and reduction in effective clot dose for stroke 3 h after clot injection (rabbit)
2006	De Taboada et al. [23]	Atherothrombotic stroke	Laser	808 nm, 7.5 mW/cm^2, 0.9 J/cm^2, 2 min per point	Improved modified neurological score at 14, 21, and 28 after MCAO (rat)
2006	Oron et al. [24]	Atherothrombotic stroke	Laser	808 nm, 7.5 mW/cm^2, 0.9 J/cm^2, 2 min per point	Improved neurological scores 14 and 21 days after MCAO; increased subventricular zone cell proliferation and migration after (rat)
2007†	Lampl et al. [15]	Ischemic stroke	Laser	808 nm, 1 J/cm^2 per point	Improved clinical outcome at 90 days after ischemic stroke (human)
2007	Lapchak et al. [25]	Embolic stroke	Laser	808 nm, 25 mW/cm^2, 15,000 J/cm^2, pulsed at 1 kHz	Improved motor function, decreased effective clot dose for stroke 6 h after clot injection (rabbit)
2007	Oron et al. [26]	Traumatic brain injury	Laser	808 nm, 10 or 20 mW/cm^2, 1.2–2.4 J/cm^2, single point for 2 min	Improved motor behavior 5 days after closed-head injury, and decreased brain lesion size from 12.1% to 1.4% at 28 days after injury (mouse)
2008*	Michalikova et al. [27]	Mild cognitive impairment, Alzheimer's disease	Laser	1072 nm, 6 min × 10 days	Improved acquisition of working memory for spatial navigation in middle-aged mice (mouse)
2008	Lapchak et al. [28]	Embolic stroke	Laser	808 nm, 25 mW/cm^2, 15,000 J/cm^2, pulsed at 1 kHz	No worsening of hemorrhage incidence, volume or survival after treatment with tPA (rabbit)
2008	Ahmed et al. [29]	Epilepsy	Laser	808 nm and 830 nm, 5.5 W/cm^2, 3.1 W/cm^2 and 2.8 W/cm^2, 30 J/point, 11 J/point and 5 J/point	Decrease in cortical aspartate, glutamate and taurine and decreased hippocampal GABA (rat)
2009†	Zivin et al. [16]	Ischemic stroke		808 nm, 1 J/cm^2 per point	No improvement in mRS or NIHSS scores, no differences in mortality or adverse events at 90 days (human)
2009	Moreira et al. [30]	Traumatic brain injury	Laser	660 nm and 780 nm, 952 mW/cm^2, 3 J/cm^2 and 5 J/cm^2	Altered interleukin and tumor necrosis factor aplpha concentrations in brain and plasma at 1 day after cryogenic brain injury (rat)
2009*†	Schiffer et al. [11]	Depression, prefrontal functions	LED	810 nm, 250 mW/cm^2, 60 J/cm^2	Decreased depression scores, increased prefrontal blood flow (human)
2010	Lapchak et al. [31]	Embolic stroke	Laser	808 nm, 25 mW/cm^2, 15,000 J/cm^2, pulsed at 1 kHz	Increased cortical ATP (rabbit)
2010	Uozumi et al. [32]	Anoxic brain injury	Laser	808 nm, 1.6 W/cm^2, 4320 J/cm^2	Increased cerebral blood flow and decreased hippocampal and cortical neuronal death after BCCAO (mouse)
2010*†	Naeser et al. [14]	Traumatic brain injury	LED	633 nm and 870 nm, 22.2 mW/cm^2, 13.3 J/cm^2	Improved cognition of 2 patients with chronic mild traumatic brain injury after 2–4 months of treatment (human)
2010	Shaw et al. [33]	Parkinson's disease	Laser	670 nm, 40 mW/cm^2, 2 J/cm^2 in four fractions	Reduction in substantia nigra dopaminergic cell loss after MPTP toxicity (mouse)
2011	Yip et al. [34]	Ischemic stroke	Laser	660 nm, 8.8 mW, 2.6 J/cm^2, 13.2 J/cm^2 and 26.4 J/cm^2, pulsed at 10 kHz	Increased expression of antiapopotic factors Akt, Bcl-2 and pBAD and decreased expression of pro-apoptotic factors caspase 3 and caspase 9 1 hr after ischemia and reperfusion induced by transient unilateral MCAO (rat)
2011*	Ando et al. [35]	Traumatic brain injury	Laser	810 nm, 50 mW/cm^2, 36 J/cm^2, continuous, pulsed, 10 Hz or 100 Hz	Improved neurological severity score and body weight; smaller lesion volumes, reduced helplessness at 4 weeks (mouse)
2011*	De Taboada et al. [20]	Alzheimer's disease	Laser	808 nm, 0.5 W/cm^2, 2.8 W/cm^2 and 5.6 W/cm^2; 675 J/cm^2, 336 J/cm^2 and 672 J/cm^2, continuous and pulsed, three fractions per week for 6 months	Decreased escape latency in Morris water maze memory task, decreased brain amyloid load and pro-inflammatory cytokines, Decreased CSF and plasma b-amyloid, increased brain ATP concentration and oxygen consumption (mouse)
2012	Quirk et al. [36]	Traumatic brain injury	LED	670 nm, 50 mW/cm^2, 15 J/cm^2, 3 or 10 daily fractions	Improved locomotor behavior, decreased pro-apoptotic and increased anti-apoptotic gene expression, increased GSH (rat)
2012	Wu et al. [37]	Traumatic brain injury	Laser	665 nm, 730 nm, 810 nm and 980 nm, 150 mW/cm^2, 36 J/cm^2, one fraction	Improved neurological severity score and accelerated neurological recovery with 665 nm and 810 nm, 4 weeks after treatment (mouse)
2012	Oron et al. [38]	Traumatic brain injury	Laser	808 nm, pulsed at 100 Hz, one fraction	Improved neurological severity score, increased survival, smaller brain infarct volumes, from 5–28 days after trauma (mouse)
2012	Khuman et al. [39]	Traumatic brain injury	Laser	800 nm, 500 mW/cm^2, 60 J/cm^2, one fraction	Improved spatial memory, decreased microglial activation two days after trauma (mouse)
2012*	Rojas et al. [4]	PTSD, specific phobia	LED	660 nm, 9 mW/cm^2, 5.4 J/cm^2, daily dosing after extinction for four days	Enhanced extinction of fear-conditioned memories, decreased renewal of conditioned-fear, increase prefrontal oxygen consumption and energy metabolism capacity (rat)
2013*†	Barrett and Gonzalez-Lima [13]	Prefrontal cognitive functions, depression	Laser	1064 nm, 250 mW/cm^2, 60 J/cm^2	Improved sustained attention/psychomotor vigilance, improved visual memory retrieval, improved affect (human)
2013	Xuan et al. [40]	Traumatic brain injury	Laser	810 nm, 25 mW/cm^2, 18 J/cm^2, 1, 3 or 14 doses	Improved neurological severity scores and wire grip and motion test scores, smaller brain lesions sizes, decreased degeneration, increased BrdU-positive cells at 14 days (mouse)
2013	Moro et al. [41]	Parkinson's disease	LED	670 nm, 5.5 mW/cm^2, 2 J/cm^2 in four fractions	Improved locomotor activity and preserved tyrosine hydroxylase-positive cells in the substantia nigra pars compacta (mouse)

Abbreviations: ATP = adenosine triphosphate, BCCAO = bilateral common carotid artery occlusion, GSH = reduced glutathione, LED = light-emitting diode, MCAO = medial cerebral artery occlusion, MPTP = 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine, mRS = modified Rankin scale, NIHSS = Neurological Institute of Health Stroke Scale, tPA = tissue plasminogen activator, * = studies testing cognitive effects, † = studies with human subjects.

wavelengths. The fundamental principle of transcranial LLLT is the delivery of photons to brain cells that are primarily absorbed by the mitochondrial respiratory enzyme cytochrome oxidase and up-regulate its enzymatic activity *in vivo* [3–5]. The proposed mechanistic rationale is that LLLT stimulation of cytochrome oxidase enhances brain oxygen utilization and metabolic capacity, which may enhance normal brain functions and protect against neurological deficits caused by reduced cerebral blood perfusion and other insults to brain energy metabolism. It is important to discuss the new data because they imply that transcranial LLLT may become a novel intervention to enhance cognitive performance and treat neurological conditions linked to mitochondrial dysfunction. In addition, no neuroscience experts have properly reviewed these findings in a detailed and integrated manner that explains how the mechanism of action of LLLT is related to both cognitive enhancement and mitochondrial neuroprotection. The current review distinguishes itself from the existing literature because it addresses the evidence of *in vivo* cognitive-enhancing effects of LLLT in the normal brain as well as the *in vivo* neuroprotective effects against neurometabolic energy failure. This review also highlights the existence of a common biochemical mechanism of action for LLLT [3] and for the mechanism of action of the metabolic enhancer and antioxidant methylene blue [6], focusing on the well-established central role of the mitochondrial enzyme cytochrome oxidase on brain function. Acknowledgment of this common mitochondrial mechanism of action is expected to provide important mechanistic insights to support the use of LLLT as a tool for the effective treatment of neurological and psychological conditions.

Photobiomodulation is the use of radiant energy to modify biological functions. LLLT is defined as the use of directional low-power and high-fluency monochromatic or quasimonochromatic light from lasers or LEDs in the red-to-near-infrared wavelengths to modulate a biological function or induce a therapeutic effect in a nondestructive and nonthermal manner [3]. The fundamental principle of photobiomodulation with LLLT is the presence of chromophores, molecules capable of absorbing light in cells and tissues. The interaction of light-excited chromophores with downstream molecules and pathways induces subsequent biochemical changes with potential pharmacological, physiological and clinical effects. LLLT with red-to-near-infrared light from lasers and LEDs may be delivered transcranially to target the brain parenchyma. Transcranial LLLT is able to modify cognitive and neurological functions in animals and humans with effects that are independent of visual pathway activation or heat [3].

2. Transcranial LLLT as a safe and novel neuromodulatory intervention

As mentioned above, the fundamental principle of photobiomodulation with LLLT is the presence of chromophores capable of absorbing light in neurons. It is well-established that cytochrome oxidase is the major neuronal photoacceptor in the red-to-near-infrared range of radiant energy, and meaningful biologic effects of LLLT in neural tissues have been documented in a number of conditions ranging from cell cultures to human subjects [3]. For example, LLLT enhances both the activity and expression of cytochrome oxidase in neurons *in vitro* [7]. Transcranial LLLT also accelerates cell respiration and energy production in the brain parenchyma *in vivo* [4,8]. In addition, LLLT partially restored enzyme activity blocked by potassium cyanide, a cytochrome oxidase inhibitor, and significantly reduced neuronal cell death induced by this mitochondrial toxin [9]. Prophylactic LLLT *in vitro* has proved very effective at protecting neurons from neurodegeneration induced by mitochondrial toxins [10]. Beneficial mitochondrial bioenergetics effects have also been demonstrated *in vivo* as LLLT-induced up-regulation of cytochrome oxidase in the cortex, when delivered transcranially [4]. Transcranial LLLT has also been observed to augment prefrontal blood flow in human subjects [11]. An encouraging common denominator of the effects of transcranial LLLT on brain cytochrome oxidase is that it is a safe intervention with null deleterious effect on the structure and function of the brain at the doses observed to induce beneficial effects [1–5]. Early investigations documented that the subunit expression and assembly of cytochrome oxidase is tightly regulated by energy consumption. Cytochrome oxidase is not only a key enzyme in oxidative metabolism, but also has a limiting step role in energy production. Cytochrome oxidase is a highly dynamic and autoinducible enzymatic complex, and it is notable for its connection with activity-dependent gene expression pathways relevant to energy metabolism, homeostasis and cell death [12]. Thus, photobiomodulation of brain cytochrome oxidase is expected to provide beneficial effects primarily *via* the up-regulation of cytochrome oxidase itself. In turn, this is expected to increase neuronal respiration and boost brain energy metabolic capacity, which would constitute an adaptation with major neuroprotective implications.

LLLT *via* commercial low-power lasers and LEDs constitutes an affordable and safe alternative to current treatment options for cognitive impairment and brain dysfunction. Low-power LED arrays and laser diode sources are compact, portable, and have achieved non-significant risk status for human trials by the FDA. High bioavailability of LLLT to brain tissue *in vivo* is supported by preclinical evidence of transcranially-induced increases in brain cytochrome oxidase activity and improved behavioral outcome in rats with impaired mitochondrial function [5] and by improved brain cytochrome oxidase activity and memory retention in normal adult rats [4]. Further evidence from the first controlled human study demonstrated the beneficial effects of transcranial infrared laser stimulation on cognitive functions [13]. Thus, LLLT treatments could be cost-effective, safe, and non-invasive [14] and could have broad impact and significance to improve the cognitive health of our growing aging population. Transcranial LLLT has already been successful at improving neurological outcome in humans in some controlled clinical trials of stroke [15,16]. However, early use of LLLT in people with compromised cerebral blood flow may prove to also be an effective strategy before stroke because its beneficial effects would be based on metabolic neuroplasticity natural to the undamaged brain, as opposed to be based on less physiologic and less generalizable processes of cell repair. In other words, LLLT has a potential as a strategy for primary or secondary stroke prevention in the specific setting of chronic brain hypoperfusion (CBH) associated with cerebrovascular atherosclerosis. Likewise, LLLT given before the onset of cognitive impairment, either vascular or associated with primary neurodegenerative processes, may induce neuroprotection by facilitating a neurochemical substrate for improved cognitive reserve. This would seem more plausible and advantageous than interruption of an advanced multifactorial neurodegenerative process in which the molecular machinery to support the secondary photobiologic effects of LLLT has been damaged. In summary, the available evidence indicates that LLLT may have the ability to enhance cognition and prevent neural dysfunction associated with CBH, stroke, traumatic brain injury, dementia and other neurodegenerative processes when given *before* the onset of brain damage.

3. Methodological considerations for transcranial LLLT

Transcranial LLLT consists of applying monochromatic light directly to the head, with wavelengths falling within an "optical window" in the red-to-near-infrared optical region (~620–1150 nm). Wavelength is a major LLLT parameter as it greatly

determines the molecular target of light [17–19]. Cytochrome oxidase shows four major light absorption peaks within the red-to-near-infrared band. These are determined by Cu_A and Cu_B, two of the four metal centers within the enzyme. These peaks of absorption are 620 nm (Cu_A reduced), 680 nm (Cu_B oxidized), 760 nm (Cu_B reduced) and 825 nm (Cu_A oxidized). *In vitro*, these absorption peaks correspond to peaks in DNA synthesis and cell attachment [17]. Within this band, light tissue penetration tends to be higher with higher wavelengths; thus, wavelengths in the upper end are preferred in transcranial applications [20–26]. However, longer wavelengths do not provide linear improvement in tissue penetration, since as the wavelengths get longer than 940 nm, light absorption by water increases [18]. Nevertheless, transcranial LLLT applications have demonstrated relevant neural and behavioral effects at wavelengths above 940 nm [13,27]. LLLT doses are expressed as *radiant exposure* in Joules (J) per surface area (J/cm^2, fluency or energy density) and radiant exposure is equivalent to power density or *irradiance* (W/cm^2) per unit of time (s). Thus for achieving a desired dose, either power density or time of exposure can be varied. However, wavelength and radiant exposure are not the only parameters that are relevant for replicating a particular photobiologic effect of LLLT, and the optimal LLLT dose may be difficult to determine for a particular application (Table 1). Besides wavelength and radiant exposure, parameters that are expected to influence the efficacy, feasibility and safety of LLLT can be of three types: (a) device parameters, (b) irradiation parameters and (c) treatment parameters [18,19]. Until further research determines how variation in such parameters will affect transcranial memory-enhancing and neuroprotective effects of LLLT, a thorough methodological description should be provided for any transcranial LLLT application. Device parameters include device manufacturer, model identifier, number of emitters, emitter type or source (*e.g.* solid state, gas, laser diode, InGa AlP LED, GaAlAs laser, KTP laser), spatial distribution of emitters and shape, size and type of beam delivery system (*e.g.* fiber optic, free air/scanned, hand-held probe). Irradiation parameters include center wavelength (nm), spectral bandwidth (nm), operating mode (*e.g.* continuous wavelength, switched continuous wavelength, pulsed), frequency (Hz), pulse duration (s), pulse off duration (s) or duty cycle (%), peak radiant power (mW), average radiant power (mW), aperture diameter (cm) and beam profile (*e.g.* Gaussian, Top Hat). Treatment parameters include beam spot size at target (cm^2), irradiance at target (mW/cm^2), exposure duration (s), radiant exposure (J/cm^2), radiant energy (J), number of points irradiated, area irradiated (cm^2), application technique (*e.g.* skin contact, contact with pressure, interstitial fiber optic), number and frequency of treatment sessions (*i.e.* number of treatments per day or week) and cumulative radiant energy (*i.e.* individual doses multiplied by the number of treatment sessions, in J) [19].

Factors such as dosing schedules and light sources have proven to be extremely relevant parameters, besides wavelength and dose. For example, delivering large total doses of LLLT in a single session produces less favorable outcomes than giving the same doses over several sessions. This dose fractionation has been tested *in vitro* and *in vivo*, and it has been shown to be highly effective at preventing neuronal degeneration. In addition, it has been also shown that LLLT fractionation protocols including prophylactic doses given before neurotoxic metabolic lesions are also effective at preventing neurodegeneration [5,10]. Similarly, light sources for LLLT may be obtained from lasers or LEDs. Laser sources produce 100% of coherent light energy in a single wavelength. They allow high tissue penetration and they produce a constant beam width that offers the advantage of energy delivery on circumscribed areas. The beam width of lasers can be modified by coupling them into fiber optic, which allows delivering energy to areas of different sizes. Yet, areas of tissues that can be treated with lasers could be insufficient for some transcranial applications, and repeated single beam exposures are usually necessary. LEDs typically have a bandwidth of 4 nm to 30 nm (at full width half maximum), and emitted light is not coherent and less collimated. Lasers are capable of delivering high amounts of energy with great efficiency, which can be high enough to rapidly produce tissue heating and damage. On the other hand, LEDs deliver energy with less efficiency, which may be advantageous in certain protocols of energy delivery. Although any light source can deliver comparable amounts of energy to a target surface, the photobiological effect will not necessarily be the same. Substitution of an LED for a laser of the same wavelength may deliver the same amount energy by varying the power or exposure time. However, this reciprocity rule has been disproven in photobiology and photomedicine [19]. In addition, with irradiances used in LLLT, LEDs generate negligible amounts of tissue heating at the site of light absorption. This reduces the risk of thermal injury [3]. In addition, LEDs can be mounted on arrays with ergonomic features that allow efficient energy delivery, which is relevant when the target organ has a large surface area, such as the brain. LED arrays and diode lasers are compact and portable, which may facilitate their use in the clinical setting, and both have achieved nonsignificant risk status for human trials by the FDA (www.clinicaltrials.gov). Finally, the importance of the operating mode of the light source has been documented in a number of studies. For example, pulsed-wave LLLT to rats is more effective at improving performance in the Morris water maze and decreasing hippocampal amyloid load than continuous wave LLLT [20].

For transcranial applications, tissue penetrance achieved with a specific light source should be ideally described. Post-mortem analyses in human specimens may provide an estimate of laser transmittance through the skull. Incident light (I_0) and transmitted light (I) should be measured with the tissue directly overlying the aperture of the detector; average readings are then used to calculate percent transmittance (k) as $100 \times I/I_0$. Optical density (OD) is calculated as—log (k). The cross-sectional width of each set of tissues may be measured with calipers, and Beer's law is then applied to calculate the absorption coefficient (a) = OD/width. Using this method, we have measured that approximately 2% of the 1064 nm wavelength at 250 mW/cm^2, 60 J/cm^2 passed through the adult human supraorbital frontal bone, when the LLLT is delivered by direct contact with bone. This yields an OD of 1.70 and an absorption coefficient of a = 0.24. This is consistent with reported values of transmittance of this wavelength through cranial bone of a = 0.22 [21]. Consequently, the transcranial LLLT dose reaching the frontal cortex surface was estimated to be 1.2 J/cm^2 [13], which is consistent with the most effective doses (around 1 J/cm^2) found to stimulate cytochrome oxidase activity in neuron cultures (reviewed in Ref. [3]). Stimulation of cognitive functions in humans has been achieved using a relatively high-wavelength 1064 nm laser diode (Cell Gen Therapeutics, LLC, Model CG-5000 laser, HD Laser Center, Dallas, TX). The wave type from this source is continuous, not pulsed. Marketing of Cell Gen lasers is FDA-cleared as safe for various indications (*e.g.* improving circulation, relief of muscle and joint pain, spasm, stiffness and relaxation). Pre-clinical studies support that the structure and function of the retina is not damaged by LLLT doses commonly used for experimental applications. In fact, such LLLT doses have been shown to exert neuroprotective effects [5]. However, scarce data is available on the potential retinotoxic effects of LLLT in humans. Thus it is advisable that subjects' eyes be covered with protective eyewear during transcranial LLLT delivery. Enhancement of cognitive and emotional functions in humans has been achieved with irradiance of 250 mW/cm^2 and fluency of 60 J/cm^2. Beneficial effects with these parameters have been observed by two independent groups [13,14]. These parameters allow transcranial

light penetration of about 2%, which corresponds to a fluency of 1.2 J/cm^2 over the cortical surface. At these power levels the energy emitted is low, exposure to it is not harmful to tissue, and it causes negligible tissue heating and no physical damage. Using these parameters, we directed a 1064 nm laser diode at the right frontal pole of the cerebral cortex [13], which is the most anterior prefrontal cortex (Brodmann's areas 9 and 10). In reference to the 10–20 system used for EEG electrode placement, the forehead stimulation site was centered on the FP1 or FP2 (left or right frontal pole) point, and extended medially and laterally for a 4 cm diameter area from this point. In animals such a rats, a power density output of 9 mW/cm^2 delivered at the 10.9 J/cm^2 dose has a transcranial transmittance of 5.8%, with 0.63 J/cm^2 reaching the rat cortical surface. With these parameters, LLLT enhanced prefrontal cortex oxygen consumption rate, increased cytochrome oxidase expression and facilitated fear-extinction memories [4].

4. *Chronological overview of* transcranial LLLT studies relevant to cognitive enhancement and neuroprotection

A sizable body of controlled studies assessing the effect of LLLT on human cognitive functions does not exist, but pioneer studies on the *in vivo* neuroprotective and cognitive-enhancing properties of LLLT started in the last decade (Table 1) [4,11,13–16,20,22–24,26–40]. Twenty-seven studies have assessed the effects of transcranial LLLT targeting the brain in healthy animals, animal models of neurological disease, healthy human subjects or patients affected by neurological disease. Five of these studies have been done with human subjects. These include one pilot case series in patients with traumatic brain injury ($n = 2$) [14], one open label, non-controlled trial in patients with depression ($n = 10$) [11], two double-blind, randomized, sham-controlled studies in patients with acute ischemic stroke ($n = 780$) [15,16] and one small placebo-controlled trial for effects on cognitive and emotional functions in healthy volunteers ($n = 40$) [13]. Seven studies have specifically tested the effects of LLLT on cognitive functions, three of them in human subjects [4,11,13,14,20,27,35]. The first published observation of LLLT's memory effects in a mouse model was the Michalikova et al. study [27]; but other than the wavelength this paper did not report other relevant LLLT parameters, making it impossible to evaluate or replicate this study. The work by De Taboada et al. [20] was the first published observation of prevention of memory loss in a mouse model of AD. This study indicated the importance of LLLT's treatment early in a disease process.

The first translational neuroprotective applications of transcranial LLLT were the NEST-1 and -2 clinical trials in stroke [15,16]. Until NEST-3 or a similar stroke clinical trial is published there is still uncertainty in the use of LLLT in stroke. So far 10 studies have assessed the effects of LLLT in ischemic, hemorrhagic, atherothrombotic, embolic or anoxic stroke. Beneficial neuroprotective effects have been observed regardless of the stroke mechanism. Functional neuroprotective effects in stroke have been correlated with changes including down-regulation of pro-apoptotic genes, up-regulation of anti-apoptotic genes, increased energy production and increased activation of cell proliferation and migration [9,42]. Nine studies have assessed the neuroprotective effects of LLLT in traumatic brain injury, and this constitutes one of the most active areas of LLLT research. The scientific literature on neuroprotective effects of LLLT in traumatic brain injury has provided vast evidence of functional, structural and cognitive effects at different time points [24,27,32,35–37,40,41]. It has also addressed the effects of wavelength, fluency, dose fraction and pulse width like no other field of *in vivo* brain photobiomodulation. Also, studies on the effects of LLLT in traumatic brain injury contain meaningful observations regarding the mechanisms through which LLLT exerts its neuroprotective effects. The available evidence shows that such neuroprotective effects may be supported by induction of cell proliferation as well as anti-oxidant, anti-inflammatory and anti-apoptotic effects. Finally experimental transcranial applications of LLLT have also explored its potential applications relevant to epilepsy, Parkinson's disease, mild cognitive impairment, Alzheimer's disease, depression and enhancement of normal cognitive function.

5. Authors' studies of transcranial LLLT effects on brain cytochrome oxidase activity, oxygen consumption and cognitive functions

Transcranial LLLT treatment and placebo effects on cytochrome oxidase and cognitive functions have been described in rats and humans in our laboratory. In 2008, Rojas et al. [5] were the first to report that upon transcranial delivery *in vivo*, LLLT induces brain metabolic and antioxidant beneficial effects measured by increases in cytochrome oxidase and superoxide dismutase. In 2011, we proposed LLLT as a novel paradigm to treat visual, neurological, and psychological conditions based on the stimulation of cytochrome oxidase activity in neurons [3]. In 2012, Rojas et al. [4] were the first to report that LLLT increased extinction memory retention and oxygen consumption in the rat frontal cortex *in vivo*. In 2013, Barrett and Gonzalez-Lima [13] reported the first controlled study of transcranial laser stimulation of psychological functions in humans. Transcranial infrared laser stimulation to the forehead has been shown to produce beneficial effects on frontal cortex measures of attention, memory and mood. Our studies have used different daily *in vivo* LLLT doses (1–60 J/cm^2), fractionation protocols (1–6 sessions), wavelengths in both the red (633 nm and 660 nm) and in the near-infrared (1064 nm), and a range of power densities (2–250 mW/cm^2). These variables allowed us to identify effective LLLT parameters for transcranial brain stimulation in rats and humans, with findings that can be summarized as follows:

LLLT increases brain oxygen consumption in vivo. An increase in cytochrome oxidase activity would be expected to facilitate oxygen consumption, as cytochrome oxidase is the enzyme that catalyzes the use of oxygen to form water in the mitochondrial electron transport chain. Thus, we tested the hypothesis that LLLT stimulates brain oxygen consumption *in vivo*. Oxygen concentration in the cortex of naïve rats was measured immediately following LLLT exposure at 9 mW/cm^2 and $\lambda = 660$ nm. The cortex oxygen concentration in control conditions (*i.e.* following no LLLT exposure) decreased only $1 \pm 0.7\%$. In contrast, LLLT induced a dose-dependent decrease in oxygen concentration of approximately $5 \pm 1\%$ after LLLT 1 J/cm^2 and $15.8 \pm 2\%$ after LLLT 5 J/cm^2. These data suggest a physiological effect of transcranial LLLT on the metabolic rate of cortical oxygen consumption [4].

LLLT induces a hormetic dose–response on brain cytochrome oxidase activity. LLLT has been shown to increase cytochrome oxidase expression in neuronal cultures [5]. It has been observed that this secondary effect of LLLT also occurs in the brain *in vivo*. The effects of different doses of transcranial LLLT were delivered in a single fraction and levels of brain cytochrome oxidase activity were measured. Unanesthetized rats were exposed to 660 nm at either 10.9 J/cm^2, 21.6 J/cm^2, 32.9 J/cm^2 or no LLLT in home cages. Treatments were delivered *via* four LED arrays with a power density of 9 mW/cm^2 for total treatment times of 20 min, 40 min and 60 min for each dose, respectively. Twenty-four hours after the single treatment session, animals were decapitated and their brains histochemically analyzed for cytochrome oxidase activity. LLLT showed enhancement of brain cytochrome oxidase following a hormetic dose–response pattern. A single dose of 10.9 J/cm^2 LLLT resulted in a 13.6% increase in cytochrome oxidase activity. In turn, a single dose of 21.6 J/cm^2 resulted in an increase of only 10.3%,

whereas the highest dose induced no significant increase in cytochrome oxidase activity (3%) [4]. A low dose given in a single day had a stimulatory effect while higher doses were less effective. Hormetic dose–response effects, such as the one demonstrated on brain cytochrome oxidase activity are not logarithmic, but LLLT repeated daily can show improvements of up to 30–60% as compared to control [3]. The available data support that although small, these effects are not negligible, but neurobiologically meaningful. It is expected that hormetic changes in brain metabolic capacity may support neurotherapeutic cognitive improvements.

LLLT increases cognitive functions in humans. LLLT has been used non-invasively in humans to stimulate the brain to improve neurological outcome after ischemic stroke [15], as an antidepressant treatment [11] as well as to alleviate muscle fatigue and enhance recovery [43]. We conducted the first controlled study demonstrating that transcranial laser stimulation enhances cognitive functions in healthy humans [13]. These LLLT treatments have thus been proven to be not just safe but actually beneficial in humans. In particular, Schiffer et al. [11] found that a single LLLT treatment to the forehead resulted in a significant beneficial effect in patients with major depression and anxiety that correlated with increased cerebral blood flow. No adverse side effects were found in any of the patients, either immediately after the initial treatment, or at 2 or 4 weeks post-treatment. We followed a similar transcranial LLLT protocol to the forehead, targeting frontal cortex-based cognitive tasks such as a psychomotor vigilance task (PVT) and a delayed match-to-sample memory task (DMS) before and after LLLT *vs.* a placebo control. The PVT is a test that assesses an individual's sustained attention. It involves the subject maintaining a vigilant state during a delay period, then responding as fast as possible when a stimulus appears onscreen. These attentional processes are mediated by frontal cortical regions and PVT has been shown to be a reliable indicator of frontal function [44]. In turn, the DMS task has been shown to be mediated by a frontoparietal network [45]. This task involves the presentation of a visual stimulus on a screen. Then the stimulus disappears, and the participant must remember the stimulus through a delay. Then two choices appear, and the participant must decide which of these two is identical to the previous stimulus (the "match").

The forehead of healthy volunteers was exposed to LLLT with continuous wave laser at λ = 1064 nm. This wavelength maximizes tissue penetration and intersects the absorption spectrum of cytochrome oxidase. The irradiance and cumulative fluency were 250 mW/cm^2 and 60 J/cm^2, respectively. These parameters are the same that showed beneficial psychological effects in the study by Schiffer et al. [11]. At the power level described, the energy emitted by the laser is low, exposure to it is not harmful to tissue, and it causes negligible heat and no physical damage. Similar settings are used clinically for treatment of chronic pain [46]. The treated group showed significant beneficial effects on the PVT. LLLT improved reaction time in the sustained vigilance test. Performance in the DMS also showed a significant improvement in treated *vs.* placebo control groups as measured by memory retrieval latency and number of correct trials [13]. These data imply that transcranial laser stimulation is effective as a noninvasive and efficacious approach to increase cognitive brain functions such as those related to attention, memory and mood.

6. Mechanisms of action of LLLT and their implications for mitochondrial neurotherapeutics

The remarkable modulatory effects of LLLT and its specific photochemical mechanisms of action have major therapeutic potential on their own, but their discovery has revealed a major and broadly generalizable therapeutic principle. The photobiomodulation effects of LLLT indicate that support of mitochondrial function is a very effective approach not only to facilitate normal cell functions, but also to preserve structural and physiological integrity in pathologic contexts. Maintenance and facilitation of optimal mitochondrial function is meaningful, since it represents a highly specialized version of a fundamental process in biological systems: assimilation and transfer of energy. Photobiomodulation is expected to have major therapeutic relevance in highly adaptable systems extremely sensitive to energy availability such as the brain.

The mechanism of action of LLLT consists of primary effects and secondary effects (Fig. 1). Primary effects occur with light on and depend on light absorption by mitochondria. The respiratory enzyme cytochrome oxidase is regarded as the major acceptor of light in the red-to-near-infrared wavelength range. Energetic improvements are expected from LLLT because it acts as an exogenous source of highly energized electrons to the respiratory chain, otherwise provided by endogenous electron donors such as NADH and $FADH_2$. This view is supported by evidence showing that LLLT facilitates the catalytic activity of cytochrome oxidase, accelerates the electron transfer in the inner mitochondrial membrane and boosts cell respiration and energy production [4,7,8,10,47]. In fact, LLLT may restore electron flow, when there is upstream blockade of electron entry into the respiratory chain [5]. In addition, because cytochrome oxidase is sensitive to energy demands, a consequence of its activation by LLLT is an increase in its subunit expression and assembly, which leads to an increase in

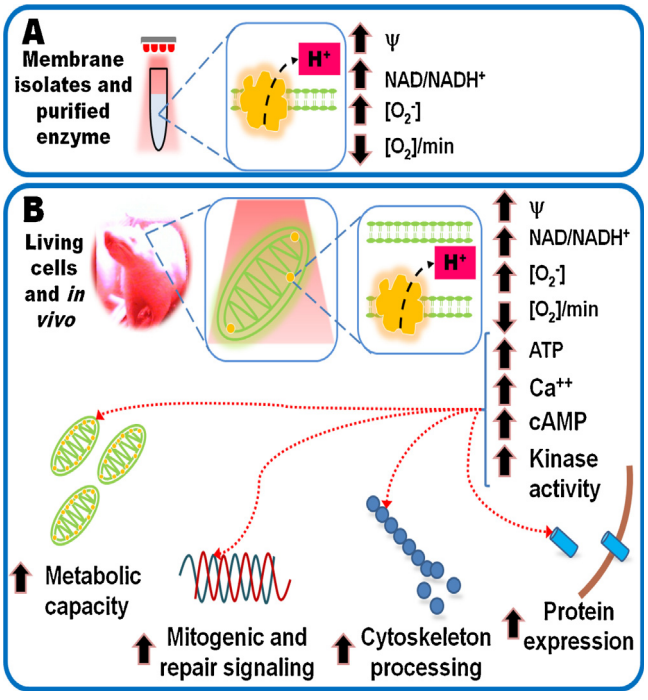

Fig. 1. Primary and secondary effects of low-level light/laser therapy (LLLT). (A) Primary effects occur with red-to-near-infrared light on and consist of direct excitation of chromophores in the respiratory enzyme cytochrome oxidase (yellow). Primary effects are fundamental for the *in vivo* beneficial effects of light therapy, but they can also be observed *in vitro* in solutions of the purified enzyme or in mitochondrial membrane isolates. The primary effects of cytochrome oxidase excitation represent a boost in the activity of the respiratory chain and consist of increases in transmembrane potential, oxidation of $NADH^+$, oxygen consumption and free radicals. (B) Secondary mechanisms may occur with light off. Secondary effects are always preceded by primary effects and they occur only in the presence of intact cellular metabolic machinery. Thus, secondary effects have been observed only in living cells and *in vivo* and not in systems of membrane or enzyme isolates. Secondary effects are pleiotropic and depend on activation of enzymatic pathways that affect metabolic capacity, gene expression for mitogenic and repair signaling, cytoskeleton processing and protein expression and translocation. Such secondary effects are triggered due to the central role of mitochondria as integrators of energy metabolism, cellular homeostasis and cell survival signaling.

neuronal oxidative metabolic capacity and photoacceptor availability [7]. In neural tissue, cytochrome oxidase is the most abundant metalloprotein, and wavelengths in its absorption spectra correlate well with its catalytic activity action spectra and with ATP content *in vitro* [5–8]. Cytochrome oxidase is a central enzyme in neuronal bioenergetics, due to its role as a rate-limiting step in ATP synthesis and its exquisite functional response to energy demands, changes in intermediate metabolism and cell damage. Cytochrome oxidase is in fact a reliable marker of neuronal energy metabolism [7]. Due to its central role in oxidative metabolism, the effects of LLLT on cytochrome oxidase are believed to be the origin of photosignal transduction from mitochondria to other neuronal compartments, including the cytoplasm, nucleus and cell membrane. These phototransduction processes beyond the respiratory chain may occur at times after light exposure and define the secondary mechanisms of LLLT. The engagement of a number of intracellular enzymatic and metabolic pathways is considered to be responsible for the pleiotropic effects of LLLT. For example, cell membrane functions, such as cell-adhesion, are susceptible to modulation *in vitro* by LLLT and this is mediated by changes in the cell surface integrin expression pattern and focal adhesion kinase activity [49]. Because no secondary LLLT effects are observed in conditions where disruption of the plasma membrane and cellular homeostasis occur, changes at the cell-membrane level are regarded as a secondary effect of LLLT, whereas the primary redox effects have been shown to occur in mitochondria. Similarly, only in conditions of cellular integrity, plasma membrane or nucleus functions are sensitive to secondary effects of LLLT [50]. Thus, the effects of LLLT ranging from light absorption to changes in neuronal function are highly dependent on the metabolic and signaling pathways available to support a photobiological response.

The relevance of the photobiochemical effects of LLLT is revealed by the fact that they are not unique to light, but they are paralleled by the neurochemical effects of methylene blue (MB), a non-photic electron donor with the ability to regulate redox and free radical processes (Table 2). MB is a redox-cycling tricyclic phenothiazine drug [48,51] that was observed to increase cell adhesion in the dark, and the magnitude of this effect was comparable to that of LLLT at $\lambda = 820$ nm at an optimal dose [49]. This observation was made, during experiments attempting to determine the role of reactive oxygen species in the photochemical effects of LLLT. In turn, inhibitors of the electron transport chain such as rotenone, dinitrophenol and sodium azide, inhibited cell adhesion, while other antioxidants such as ascorbic acid and melatonin, had no effect on cell-adhesion. MB added to the cells in the dark also caused stimulation of DNA synthesis at a percentage comparable with the stimulation caused by LLLT. MB has unique metabolic-enhancing effects and antioxidant properties that are superior to other redox compounds [6]. In fact, MB has been recognized to have one of the most potent chain-breaking antioxidant profiles [51]. Unlike most conventional short-lived radical traps, MB has the potential to autoxidize, which means that its reduction–oxidation capacity allows electron cycling, without MB gaining any permanent stoichiometric or net reduction. Thus, depending on the medium redox state and pH, MB can display a remarkable effect: the transfer of electrons to oxygen or alternate electron acceptors. In this manner, MB may act as an electron shuttle in the respiratory chain (Fig. 2). Taking such MB properties into account, three mechanistic similarities between LLLT and MB in their beneficial effects on the brain may be designated. These include (1) neuroprotection and memory-improving effects mediated by enhancement of neuronal oxidative metabolic capacity at the level of the respiratory chain, (2) pharmacologic hormetic dose–response curves, and (3) enhancing effects that show brain region activational specificity.

6.1. Enhancement of the respiratory chain

First, similar to the action of LLLT, MB also increases cytochrome oxidase activity *in vitro*, and enhances its expression in the brain *in vivo* [4]. Second, similar to LLLT, MB may restore electron flow in systems inhibited upstream in the respiratory chain by the complex I inhibitor rotenone [52]. Due to these effects, MB has been classically used as an artificial electron donor in early experiments of cell respiration. Reduction of coenzyme Q and cytochrome c, increases in NADH oxidation by mitochondria [53] and increases in ATP synthesis [54] support a direct effect of MB on the electron transport chain, similar to the primary effects of LLLT. Third, MB has been shown to impact downstream metabolic process in a pleiotropic fashion, emulating the secondary effects of LLLT. MB is able to stimulate glucose metabolism in anoxic conditions [54], glycolysis and Na^+/K^+ ATPase activity [55]. Both MB and LLLT have also shown neuroprotective effects against mitochondrial dysfunction in the retina *in vivo* [5,52,56] and in transgenic mouse models of Amyloid β peptide brain amyloidosis

Table 2
Similar properties and effects of low-level light/laser therapy and methylene blue relevant for neuroprotective and cognitive-enhancing applications[a].

Properties/effects	Low-level light/laser therapy	Low-dose methylene blue
Brain cytochrome oxidase	Increased expression *in vivo*	Increased expression *in vivo*
Mechanism of action	*Primary*: enhancement of cell respiration, reactive oxygen species, photon donor *Secondary*: pleiotropic	*Primary*: enhancement of cell respiration, antioxidant, electron shuttle, electron donor, *Secondary*: pleiotropic
Bioavailability	2–10% of energy delivered transcranially may reach the cortex	Crosses the blood-brain barrier, concentrates in nervous tissue, and localizes to mitochondria
Conditions affecting brain effects	Redox and activational status of target tissue, fluency, irradiance, wavelength, number of fraction, pulse width	Redox and activational status of target tissue, mg/kg dose, local or systemic administration (oral, intravenous, intraperitoneal)
Dose–response curve	Hormesis documented	Hormesis documented
Memory enhancing effects	Improved spatial working memory and fear extinction. Improved spatial memory in transgenic mouse models of amyloid dysfunction and models of traumatic brain injury	Improved spatial memory and fear extinction, inhibitory avoidance, object recognition, open field habituation. Rescues memory function in models of amnestic mild cognitive impairment induced by mitochondrial dysfunction and anticholinergics. Improved spatial memory in transgenic mouse model of amyloid dysfunction
Neuroprotective effects in animal models	Ischemic models, neurotrauma models, neurotoxicity models, Alzheimer's and Parkinson's disease models	Ischemic models, neurotrauma models, neurotoxicity models, Alzheimer's and Parkinson's disease models
Effects in controlled clinical trials with humans	Improved neurological outcome after stroke. Improved psychomotor vigilance, visual memory retrieval, executive function, inhibition, and inhibition accuracy	Reversal of ifosfamide-induced encephalopathy. Improved psychological symptoms in bipolar and unipolar depressive disorders and Alzheimer's patients

[a] Reviewed in text and in more detail in Refs. [1–6].

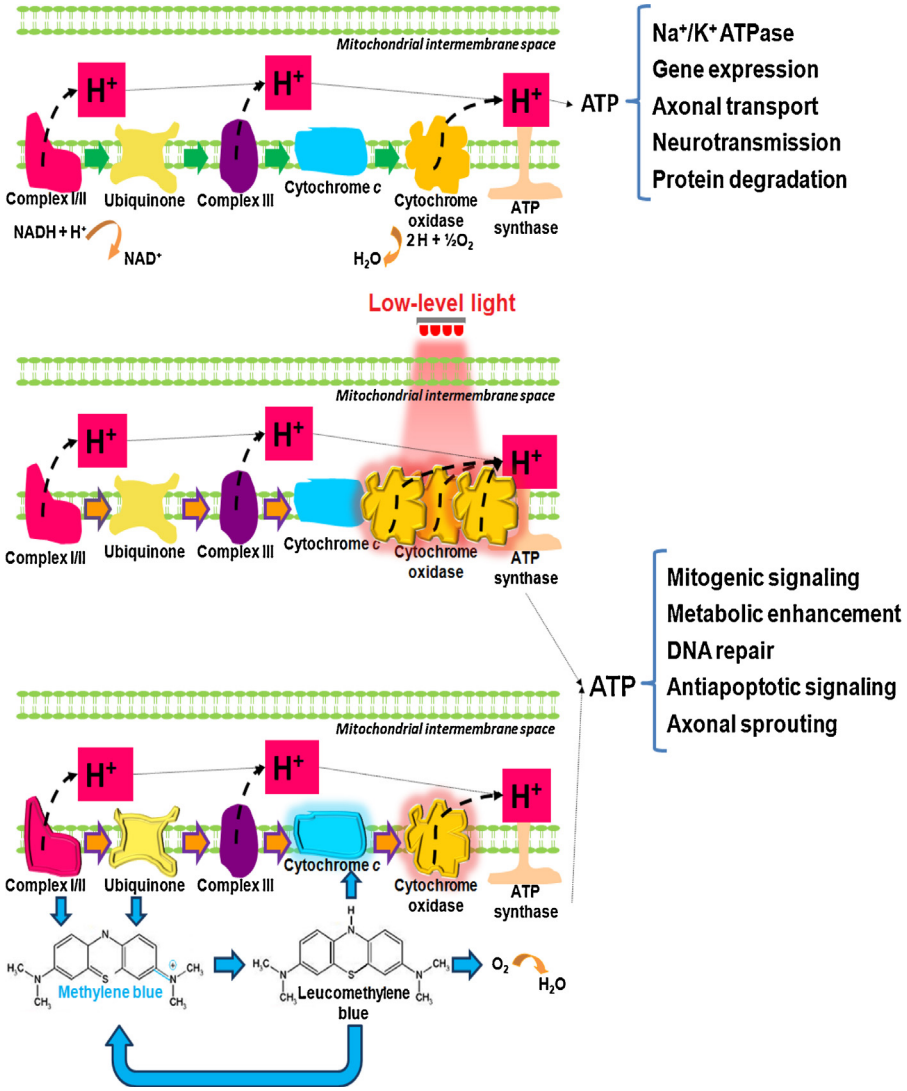

Fig. 2. Enhancement of the mitochondrial respiratory chain as the basis for cognitive enhancement and neuroprotection. Two different strategies, low-level light/laser therapy and methylene blue can achieve neuroprotective and cognitive enhancing effects by supporting and improving cell respiration. High-energy electrons are feed to the mitochondrial respiratory chain by endogenous electron donors such as $NADH^+$, which interacts with complex I or $FADH_2^+$, which interacts with complex II. Electrons flow to ubiquinone, and subsequently to complex III, cytochrome *c* and finally complex IV (cytochrome oxidase). During this transfer, electrons release energy in a tightly regulated fashion, which allows the pumping of protons into the mitochondrial inter-membrane space. This allows the storage of energy as an electrochemical gradient that is used in the synthesis of ATP (top panel). Both low-level red-to-near-infrared light and methylene blue improve cell respiration. Low-level light directly stimulates cytochrome oxidase, facilitating its catalytic activity and inducing an increase in holloenzyme subunit assembly, which improves neuronal metabolic capacity (mid panel). Similarly, methylene blue acts as an exogenous electron shuttle, also boosting cell respiration and inducing changes that improve mitochondrial metabolic capacity (bottom panel). Both interventions may have a higher facilitating effect of cell respiration in those neurons with increased energy demands, conditionally engaging and improving mechanisms required in cognitive processing and neuroprotection.

[57,58]. Finally, the notable similarities between LLLT and MB are also evident as their ability to enhance cognitive function. As discussed above, LLLT has been used in rats to improve spatial working memory [27], decrease helplessness scores [35] and facilitate fear extinction [4]. In humans, LLLT decreases depression-related scores [13], and improves psychomotor vigilance, visual memory retrieval, executive function, inhibition, and inhibition accuracy [13,14]. Animal studies have documented memory-enhancing properties in fear extinction using both LLLT and MB. MB has also shown memory-enhancing effects in a number of learning and memory paradigms including inhibitory avoidance, spatial memory, fear extinction, object recognition, open-field habituation and discrimination learning [4]. In addition, MB rescues memory function in models of amnestic mild cognitive impairment induced by mitochondrial dysfunction [59,60] or anticholinergics [61] and improves memory in transgenic mouse models of amyloid-associated memory dysfunction [57,58]. These observations and the evidence discussed above support that the mechanistic similarities between LLLT and MB are generalizable, and that support of the electron transport chain may have broad potential neuroprotective and cognitive-enhancing applications.

6.2. Hormesis

The hormetic response of both LLLT and MB consists of an increase in the effect at a low dose, followed by a decrease in the same effect with an intermediate dose, until the effect is equal to a control-type effect. With doses increasing beyond the hormetic zone, the effect decreases even further, until it is below the control effect. Both interventions induce maximal pharmacologic effects that correspond to 30–60% increases compared to control, as opposed to several fold-increases typical of linear-non-threshold

dose–response curves [19]. The magnitude of such effects is typical of hormesis, and they have been considered rare and negligible by classical pharmacology paradigms but it is known now that they are very common, and biologically relevant [62]. Hormetic effects for both LLLT and MB at the neurochemical and behavioral levels have been described [63,64]. In particular, LLLT and MB increase brain cytochrome oxidase activity in a hormetic dose–response manner. If the principle of hormesis is generalizable in neurotherapeutic applications, lower doses of interventions that support mitochondrial function will induce increased beneficial effects, compared to higher doses.

6.3. Brain region activational specificity

Experimental evidence that the effects of LLLT and MB show brain region activational specificity has been provided by studies of facilitation of conditioned-fear extinction in rats [2]. LLLT given during the period of memory consolidation induced facilitation of fear extinction memory, which is known to be mediated by increased metabolic activity in the prefrontal cortex. When *in situ* oxygen consumption and cytochrome oxidase activity were measured in the prefrontal cortex, subjects treated with LLLT showed increases in both parameters compared to untreated controls. Similarly, MB given during the memory consolidation phase of fear extinction was correlated with selective increases of cytochrome oxidase activity in the prefrontal cortex. LLLT is more susceptible to be absorbed by a mixed valence cytochrome oxidase (*i.e.* partially reduced or oxidized). The probability of finding a mixed valence enzyme is higher with higher respiratory chain electron flow, a state that is found in highly metabolic active tissues. Similarly, MB has been described as a "magic bullet", as it concentrates in areas with high redox activity. Due to its affinity for active oxidoreductases, MB has the greatest bioavailability to mitochondria with high rates of electron transfer. Thus, both treatments may reach the totality of the brain, but only those areas that show higher metabolic rates will maximally benefit from the effects of these interventions. These areas are likely to contain neuronal networks engaged in a particular cognitive task. Thus, if the activational specificity principle is generalizable, it is expected that mitochondrial interventions will provide the greatest benefit when paired with physical therapy, cognitive rehabilitation or any other strategy that would engage regional brain energy metabolism activation.

7. Future potential role of LLLT in the treatment of neurodegeneration and cognitive impairment

There is a compelling public health need to develop interventions to prevent and effectively treat neuropsychological diseases. The burden of memory deficits in the aging population, including those at risk for developing mild cognitive impairment (MCI), Alzheimer's disease (AD) and stroke is especially important, since it is expected to reach unparalleled endemic proportions. In the US, between 2.4 and 5.1 million people may have AD with enormous personal and societal costs, whereas stroke is the third leading cause of death and the leading cause of long-term disability, with $ 43 billion cost on stroke patient care (NIH) [65,66]. There is a lack of disease-modifying treatments, and it is critical to intervene early in the natural history of neurodegeneration, ideally before the onset of cognitive impairment or severe neurological deficits. For such reasons accessible strategies to stimulate the brain, enhance its performance and prevent cognitive and neurological deficits are one of the most important research priorities of our times. Interventions that boost the cognitive reserve in healthy individuals may play a major role in the effective management of chronic neurological dysfunction associated with AD and stroke. While multiple mechanisms are likely responsible for MCI and AD, there is no question that CBH secondary to cerebrovascular atherosclerotic steno-occlusive disease and inhibition of the mitochondrial enzyme cytochrome oxidase are metabolic risk factors for MCI and AD, as well as for vascular dementia and stroke [67]. Thus, it has been hypothesized that the adverse cognitive consequences of CBH may be modifiable to prevent or delay amnestic MCI and neurodegeneration [68]. The apparent link between age-related cognitive decline, CBH and mitochondrial dysfunction has been deciphered by basic and clinical research in the last 30 years. On one hand, a strong body of evidence supports a role of mitochondrial dysfunction in memory-related neurodegenerative disorders. In addition, mitochondrial dysfunction and the concomitant oxidative stress and energy hypometabolism are believed to play a role in CBH-induced neuropathology [69]. For example, it is well-established that the brain, and in particular the aging brain, is vulnerable to hypoperfusion because it depends almost exclusively on electron transport-derived oxidative energy [70]. Regional cytochrome oxidase dysfunction has been observed in brains of patients affected by MCI and AD [67]. Cytochrome oxidase has a key role in neuronal activity as the rate-limiting enzyme for oxidative energy production in the mitochondrial electron transport and it also can catalyze the production of nitric oxide under hypoxic conditions [71]. Since memory functions are extremely sensitive to oxidative energy deficits, cytochrome oxidase inhibition linked to aging and impairment in cerebral perfusion has been proposed as a major pathophysiological mechanism underlying memory dysfunction and neurodegeneration. Recent neuroimaging evidence, in particular with arterial spin labeling fMRI techniques, have established that CBH in the elderly is associated with cognitive decline [72], is present prior to AD onset [73] and can identify patients with high risk conversion from healthy aging to MCI to AD [74].

Surprisingly, the overwhelming evidence supporting cytochrome oxidase as an ideal molecular target to promote neuroprotection and memory enhancement has not been utterly exploited in translational medicine. Specifically, no preclinical model or clinical protocol has ever investigated if improving brain cytochrome oxidase activity may prevent memory impairment or neurological decline caused by CBH. In particular, more research is needed to document *in vivo* LLLT effects on hypoxic CBH conditions in which cytochrome oxidase may catalyze the synthesis of nitric oxide from nitrite, a biochemical process different from the classic nitric oxide synthase enzymes [75]. The cognitive decline that unfolds in the general aging population, as well as in patients with MCI, AD and vascular dementia associated with CBH may be prevented by LLLT interventions that critically influence cognition, provide neuroprotection or enhance neural cell repair. The LLLT approach is scientifically relevant because it will take the research community toward translational, noninvasive, accessible and early interventions to modify the risk factors affecting the cognitive and neurological health of our growing aging population.

8. Concluding remarks

It is expected that research on transcranial applications of LLLT for neuroprotection and cognitive enhancement, especially in human subjects, will increase in the forthcoming years. Further LLLT research should go beyond preclinical and clinical experimental testing of LLLT effects. Specifically, there is a need to further test the proposed mechanistic causality between stimulation of cytochrome oxidase with LLLT and its improvement of cognitive functions. The hypothesis that a primary molecular mechanism of action of LLLT on cognitive deficits is caused by up-regulation of cytochrome oxidase needs further validation. This may be accomplished in animal models using comparisons with LLLT-treated and

untreated control groups where cytochrome oxidase activity will be chronically stimulated or inhibited, as well as testing other light wavelengths that are not absorbed by cytochrome oxidase. In addition, there is a need to evaluate the hypothesis that LLLT will inhibit the direct pathophysiologic consequences of CBH, through proteomic quantification of markers of oxidative stress, fMRI measures of cerebral blood flow, blood oxygen level-dependent signaling and cerebral metabolic rate of oxygen consumption. Similarly, the effect of early LLLT in the pathophysiology of pre-symptomatic cognitive decline may be assessed by measuring its effects on chemical and functional predictors of progression such as imaging-based glucose metabolism functional connectivity and blood biomarker levels, among others. Future research should also focus on a more extensive description of the neurotherapeutic effects of LLLT based on its dosimetry-related parameters, as well as on further elucidation of the secondary mechanisms of action (*i.e.* long-lasting cellular effects that occur once light is off) that are critical for neuromodulation. Such studies are relevant to the secondary mechanisms of action of LLLT given its documented effects on nitric oxide production and its relationship with cytochrome oxidase activity modulation [3]. Finally, it is anticipated that accelerated progress in the field of LLLT for neurotherapeutic applications will derive from a better understanding of how such therapeutic photobiological effects can be modulated by concomitant pharmacotherapy, psychotherapy and physical and cognitive rehabilitation.

Non-invasive LLLT appears to be a safe and convenient tool for mitochondrial enhancement and together with other strategies to augment cell respiration may be part of a comprehensive approach for treatment of neurological conditions featuring neurodegeneration and cognitive impairment. Support of energy metabolism at the mitochondrial level may be a fundamental neurotherapeutic strategy. The bioenergetic particularities of the brain demand consideration of non-conventional strategies of neuroprotection and enhancement, with attention to very specific neuropharmacologic details to ensure maximal efficacy. Acknowledgment of the fundamental role of oxidative metabolism and its tremendous potential as a neurotherapeutic target is desirable and may be the necessary step to advance treatments in clinical neuroscience, which has traditionally lacked the benefit of disease modifying therapies. Targeted redox-mediated bioenergetic neuromodulation with LLLT is proposed as part of a holistic neurotherapeutic construct that focuses on optimizing both the neural context (*e.g.* aerobic exercise, rehabilitation, cognitive therapy) and the redox-energy equilibrium through increases of energy availability (*e.g.* cardiovascular risk factor reduction, ketogenic diet) and mitochondrial respiration (*e.g.* LLLT, MB), as well as rationalized reduction of the pro-oxidant tendencies of neurobiological systems (*e.g.* MB, other exogenous or endogenous antioxidants). The crossroads between modern photobiology with lasers and LEDs and bioenergetics has the potential to lead a revolution in the way we treat brain dysfunction and enhance cognition.

Acknowledgments

We thank Dr. Douglas Barrett for helping with the graphical abstract.

References

[1] Lampl Y. Laser treatment for stroke. Expert Rev Neurother 2007;7:961–5.
[2] Hashmi JT, Huang YY, Osmani BZ, Sharma SK, Naeser MA, Hamblin MR. Role of low-level laser therapy in neurorehabilitation. PM&R 2010;2:S292–305.
[3] Rojas JC, Gonzalez-Lima F. Low-level light therapy of the eye and brain. Eye Brain 2011;3:49–67.
[4] Rojas JC, Bruchey AK, Gonzalez-Lima F. Low-level light therapy improves cortical metabolic capacity and memory retention. J Alzheimers Dis 2012;32:741–52.
[5] Rojas JC, Lee J, John JM, Gonzalez-Lima F. Neuroprotective effects of near-infrared light in an in vivo model of mitochondrial optic neuropathy. J Neurosci 2008;28:13511–21.
[6] Rojas JC, Bruchey AK, Gonzalez-Lima F. Neurometabolic mechanisms for memory enhancement and neuroprotection of methylene blue. Prog Neurobiol 2012;96:32–45.
[7] Wong-Riley MT, Liang HL, Eells JT, Chance B, Henry MM, Buchmann E, et al. Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome *c* oxidase. J Biol Chem 2005;280:4761–71.
[8] Mochizuki-Oda N, Kataoka Y, Cui Y, Yamada H, Heya M, Awazu K. Effects of near-infra-red laser irradiation on adenosine triphosphate and adenosine diphosphate contents of rat brain tissue. Neurosci Lett 2002;323:207–10.
[9] Liang HL, Whelan HT, Eells JT, Meng H, Buchmann E, Lerch-Gaggl A, et al. Photobiomodulation partially rescues visual cortical neurons from cyanide-induced apoptosis. Neuroscience 2006;139:639–49.
[10] Ying R, Liang HL, Whelan HT, Eells JT, Wong-Riley MT. Pretreatment with near-infrared light via light-emitting diode provides added benefit against rotenone- and MPP+-induced neurotoxicity. Brain Res 2008;1243:167–73.
[11] Schiffer F, Johnston AL, Ravichandran C, Polcari A, Teicher MH, Webb RH, et al. Psychological benefits 2 and 4 weeks after a single treatment with near infrared light to the forehead: a pilot study of 10 patients with major depression and anxiety. Behav Brain Funct 2009;5:46.
[12] Wong-Riley MT, Nie F, Hevner RF, Liu S. Brain cytochrome oxidase: functional significance and bigenomic regulation in the CNS. In: Gonzalez-Lima F, editor. Cytochrome oxidase in neuronal metabolism and Alzheimer's disease. New York: Springer; 1998. p. 1–53.
[13] Barrett DW, Gonzalez-Lima F. Transcranial infrared laser stimulation produces beneficial cognitive and emotional effects in humans. Neuroscience 2013;230:13–23.
[14] Naeser MA, Saltmarche A, Krengel MH, Hamblin MR, Knight JA. Improved cognitive function after transcranial, light-emitting diode treatments in chronic, traumatic brain injury: two case reports. Photomed Laser Surg 2010;29:351–8.
[15] Lampl Y, Zivin JA, Fisher M, Lew R, Welin L, Dahlof B, et al. Infrared laser therapy for ischemic stroke: a new treatment strategy: results of the NeuroThera Effectiveness and Safety Trial-1 (NEST-1). Stroke 2007;38:1843–9.
[16] Zivin JA, Albers GW, Bornstein N, Chippendale T, Dahlof B, Devlin T, et al. Effectiveness and safety of transcranial laser therapy for acute ischemic stroke. Stroke 2009;40:1359–64.
[17] Karu TI, Pyatibrat LV, Kolyakov SF, Afanasyeva NI. Absorption measurements of a cell monolayer relevant to phototherapy: reduction of cytochrome *c* oxidase under near IR radiation. J Photochem Photobiol B 2005;81:98–106.
[18] Chung H, Dai T, Sharma SK, Huang YY, Carroll JD, Hamblin MR. The nuts and bolts of low-level laser (light) therapy. Ann Biomed Eng 2012;40:516–33.
[19] Jenkins PA, Carroll JD. How to report low-level laser therapy (LLLT)/photomedicine dose and beam parameters in clinical and laboratory studies. Photomed Laser Surg 2011;29:785–7.
[20] De Taboada L, Yu J, El-Amouri S, Gattoni-Celli S, Richieri S, McCarthy T, et al. Transcranial laser therapy attenuates amyloid-beta peptide neuropathology in amyloid-beta protein precursor transgenic mice. J Alzheimers Dis 2011;23:521–35.
[21] Bashkatov AN, Genina EA. Optical properties of human cranial bone in the spectral range from 800 to 2000 nm. Proc SPIE 2006;6163:61630.
[22] Lapchak PA, Wei J, Zivin JA. Transcranial infrared laser therapy improves clinical rating scores after embolic strokes in rabbits. Stroke 2004;35:1985–8.
[23] De Taboada L, Ilic S, Leichliter-Martha S, Oron U, Oron A, Streeter J. Transcranial application of low-energy laser irradiation improves neurological deficits in rats following acute stroke. Lasers Surg Med 2006;38:70–3.
[24] Oron A, Oron U, Chen J, Eilam A, Zhang C, Sadeh M, et al. Low-level laser therapy applied transcranially to rats after induction of stroke significantly reduces long-term neurological deficits. Stroke 2006;37:2620–4.
[25] Lapchak PA, Salgado KF, Chao CH, Zivin JA. Transcranial near-infrared light therapy improves motor function following embolic strokes in rabbits: an extended therapeutic window study using continuous and pulse frequency delivery modes. Neuroscience 2007;148:907–14.
[26] Oron A, Oron U, Streeter J, de Taboada L, Alexandrovich A, Trembovler V, et al. Low-level laser therapy applied transcranially to mice following traumatic brain injury significantly reduces long-term neurological deficits. J Neurotrauma 2007;24:651–6.
[27] Michalikova S, Ennaceur A, van Rensburg R, Chazot PL. Emotional responses and memory performance of middle-aged CD1 mice in a 3D maze: effects of low infrared light. Neurobiol Learn Mem 2008;89:480–8.
[28] Lapchak PA, Han MK, Salgado KF, Streeter J, Zivin JA. Safety profile of transcranial near-infrared laser therapy administered in combination with thrombolytic therapy to embolized rabbits. Stroke 2008;39:3073–8.
[29] Ahmed NA, Radwan NM, Ibrahim KM, Khedr ME, El Aziz MA, Khadrawy YA. Effect of three different intensities of infrared laser energy on the levels of amino acid neurotransmitters in the cortex and hippocampus of rat brain. Photomed Laser Surg 2008;26:479–88.
[30] Moreira MS, Velasco IT, Ferreira LS, Ariga SK, Barbeiro DF, Meneguzzo DT, et al. Effect of phototherapy with low intensity laser on local and systemic immunomodulation following focal brain damage in rat. J Photochem Photobiol B 2009;97:145–51.
[31] Lapchak PA, De Taboada L. Transcranial near infrared laser treatment (NILT) increases cortical adenosine-5′-triphosphate (ATP) content following embolic strokes in rabbits. Brain Res 2010;1306:100–5.

[32] Uozumi Y, Nawashiro H, Sato S, Kawauchi S, Shima K, Kikuchi M. Targeted increase in cerebral blood flow by transcranial near-infrared laser irradiation. Lasers Surg Med 2010;42:566–76.
[33] Shaw VE, Spana S, Ashkan K, Benabid AL, Stone J, Baker GE, et al. Neuroprotection of midbrain dopaminergic cells in MPTP-treated mice after near-infrared light treatment. J Comp Neurol 2010;518:25–40.
[34] Yip KK, Lo SC, Leung MC, So KF, Tang CY, Poon DM. The effect of low-energy laser irradiation on apoptotic factors following experimentally induced transient cerebral ischemia. Neuroscience 2011;190:301–6.
[35] Ando T, Xuan W, Xu T, Dai T, Sharma SK, Kharkwal GB, 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:e26212.
[36] Quirk BJ, Torbey M, Buchmann E, Verma S, Whelan HT. Near-infrared photobiomodulation in an animal model of traumatic brain injury: improvements at the behavioral and biochemical levels. Photomed Laser Surg 2012;30:523–9.
[37] Wu Q, Xuan W, Ando T, Xu T, Huang L, Huang YY, et al. Low-level laser therapy for closed-head traumatic brain injury in mice: effect of different wavelengths. Lasers Surg Med 2012;44:218–26.
[38] Oron A, Oron U, Streeter J, De Taboada L, Alexandrovich A, Trembovler V, et al. Near infrared transcranial laser therapy applied at various modes to mice following traumatic brain injury significantly reduces long-term neurological deficits. J Neurotrauma 2012;29:401–7.
[39] Khuman J, Zhang J, Park J, Carroll JD, Donahue C, Whalen MJ. Low-level laser light therapy improves cognitive deficits and inhibits microglial activation after controlled cortical impact in mice. J Neurotrauma 2012;29:408–17.
[40] Xuan W, Vatansever F, Huang L, Wu Q, Xuan Y, Dai T, et al. Transcranial low-level laser therapy improves neurological performance in traumatic brain injury in mice: effect of treatment repetition regimen. PLoS One 2013;8:e53454.
[41] Moro C, Torres N, El Massri N, Ratel D, Johnstone DM, Stone J, et al. Photobiomodulation preserves behaviour and midbrain dopaminergic cells from MPTP toxicity: evidence from two mouse strains. BMC Neurosci 2013;14:40.
[42] Byrnes KR, Wu X, Waynant RW, Ilev IK, Anders JJ. Low power laser irradiation alters gene expression of olfactory ensheathing cells in vitro. Lasers Surg Med 2005;37:161–71.
[43] Leal Junior EC, Lopes-Martins RA, Frigo L, De Marchi T, Rossi RP, de Godoi V, et al. Effects of low-level laser therapy (LLLT) in the development of exercise-induced skeletal muscle fatigue and changes in biochemical markers related to postexercise recovery. J Orthop Sports Phys Ther 2010;40:524–32.
[44] Drummond SP, Bischoff-Grethe A, Dinges DF, Ayalon L, Mednick SC, Meloy MJ. The neural basis of the psychomotor vigilance task. Sleep 2005;28:1059–68.
[45] Nieder A, Miller EK. A parieto-frontal network for visual numerical information in the monkey. PNAS 2004;101:7457–62.
[46] Jang H, Lee H. Meta-analysis of pain relief effects by laser irradiation on joint areas. Photomed Laser Surg 2012;30:405–17.
[47] Pastore D, Greco M, Passarella S. Specific helium-neon laser sensitivity of the purified cytochrome c oxidase. Int J Radiat Biol 2000;76:863–70.
[48] Schirmer RH, Adler H, Pickhardt M, Mandelkow E. Lest we forget you—methylene blue. . .. Neurobiol Aging 2011;32. 2325.e7–16.
[49] Karu TI, Pyatibrat LV, Kalendo GS. Cell attachment modulation by radiation from a pulsed light diode (lambda = 820 nm) and various chemicals. Lasers Surg Med 2001;28:227–36.
[50] Karu TI, Pyatibrat LV, Kalendo GS, Esenaliev RO. Effects of monochromatic low-intensity light and laser irradiation on adhesion of HeLa cells in vitro. Lasers Surg Med 1996;18:171–7.
[51] Ohlow MJ, Moosmann B. Phenothiazine: the seven lives of pharmacology's first lead structure. Drug Discovery Today 2011;16:119–31.
[52] Zhang X, Rojas JC, Gonzalez-Lima F. Methylene blue prevents neurodegeneration caused by rotenone in the retina. Neurotoxicity Res 2006;9:47–57.
[53] Gabrielli D, Belisle E, Severino D, Kowaltowski AJ, Baptista MS. Binding, aggregation and photochemical properties of methylene blue in mitochondrial suspensions. Photochem Photobiol 2004;79:227–32.
[54] Lee RB, Urban JP. Functional replacement of oxygen by other oxidants in articular cartilage. Arthritis Rheum 2002;46:3190–200.
[55] Furian AF, Fighera MR, Oliveira MS, Ferreira AP, Fiorenza NG, de Carvalho Myskiw J, et al. Methylene blue prevents methylmalonate-induced seizures and oxidative damage in rat striatum. Neurochem Int 2007;50:164–71.
[56] Rojas JC, John JM, Lee J, Gonzalez-Lima F. Methylene blue provides behavioral and metabolic neuroprotection against optic neuropathy. Neurotoxicity Res 2009;15:260–73.
[57] Medina DX, Caccamo A, Oddo S. Methylene blue reduces abeta levels and rescues early cognitive deficit by increasing proteasome activity. Brain Pathol 2011;21:140–9.
[58] O'Leary 3rd JC, Li Q, Marinec P, Blair LJ, Congdon EE, Johnson AG, et al. Phenothiazine-mediated rescue of cognition in tau transgenic mice requires neuroprotection and reduced soluble tau burden. Mol Neurodegener 2010;5:45.
[59] Callaway NL, Riha PD, Wrubel KM, McCollum D, Gonzalez-Lima F. Methylene blue restores spatial memory retention impaired by an inhibitor of cytochrome oxidase in rats. Neurosci Lett 2002;332:83–6.
[60] Riha PD, Rojas JC, Gonzalez-Lima F. Beneficial network effects of methylene blue in an amnestic model. Neuroimage 2011;54:2623–34.
[61] Deiana S, Harrington CR, Wischik CM, Riedel G. Methylthioninium chloride reverses cognitive deficits induced by scopolamine: comparison with rivastigmine. Psychopharmacology 2009;202:53–65.
[62] Calabrese EJ. Hormesis: principles and applications for pharmacology and toxicology. Am J Pharmacol Toxicol 2008;3:59–71.
[63] Bruchey AK, Behavioral Gonzalez-Lima F, Physiological. Biochemical hormetic responses to the autoxidizable dye methylene blue. Am J Pharmacol Toxicol 2008;3:72–9.
[64] Huang YY, Sharma SK, Carroll J, Hamblin MR. Biphasic dose response in low level light therapy—an update. Dose–Response (a publication of International Hormesis Society) 2011;9:602–18.
[65] National Institute of Neurological Disorders and Stroke. Stroke: hope through research. National Institutes of Health; 2004.
[66] National Institute of Aging. Alzheimer's disease progress report: intensifying the research effort. U.S. Department of Health & Human Services, NIH; 2012.
[67] Arntzen KA, Schirmer H, Johnsen SH, Wilsgaard T, Mathiesen EB. Carotid atherosclerosis predicts lower cognitive test results: a 7-year follow-up study of 4371 stroke-free subjects—the Tromso study. Cerebrovasc Dis 2012;33:159–65.
[68] Valla J, Berndt JD, Gonzalez-Lima F. Energy hypometabolism in posterior cingulate cortex of Alzheimer's patients: superficial laminar cytochrome oxidase associated with disease duration. J Neurosci 2001;21:4923–30.
[69] de la Torre JC. Cardiovascular risk factors promote brain hypoperfusion leading to cognitive decline and dementia. Cardiovasc Psychiatry Neurol 2012;2012:367516.
[70] Sokoloff L, Siegel BW, Agranoff BW, Albers RW, Molinoff P. Circulation and energy metabolism of the brain. Boston: Little Brown; 1989.
[71] Poyton RO, Castello PR, Ball KA, Woo DK, Pan N. Mitochondria and hypoxic signaling: a new view. Ann NY Acad Sci 2009;1177:48–56.
[72] Bangen KJ, Restom K, Liu TT, Wierenga CE, Jak AJ, Salmon DP, et al. Assessment of Alzheimer's disease risk with functional magnetic resonance imaging: an arterial spin labeling study. J Alzheimers Dis 2012;31(Suppl 3):S59–74.
[73] Johnson NA, Jahng GH, Weiner MW, Miller BL, Chui HC, Jagust WJ, et al. Pattern of cerebral hypoperfusion in Alzheimer disease and mild cognitive impairment measured with arterial spin-labeling MR imaging: initial experience. Radiology 2005;234:851–9.
[74] Mak HK, Chan Q, Zhang Z, Petersen ET, Qiu D, Zhang L, et al. Quantitative assessment of cerebral hemodynamic parameters by QUASAR arterial spin labeling in Alzheimer's disease and cognitively normal elderly adults at 3-tesla. J Alzheimers Dis 2012;31:33–44.
[75] Poyton RO, Ball KA. Therapeutic photobiomodulation: nitric oxide and a novel function of mitochondrial cytochrome *c* oxidase. Discov Med 2011;11:154–9.