Melinda Fitzgerald*, Stuart Hodgetts, Corinna Van Den Heuvel, Riccardo Natoli, Nathan S. Hart, Krisztina Valter, Alan R. Harvey, Robert Vink, Jan Provis and Sarah A. Dunlop

Red/near-infrared irradiation therapy for treatment of central nervous system injuries and disorders

Abstract: Irradiation in the red/near-infrared spectrum (R/NIR, 630–1000 nm) has been used to treat a wide range of clinical conditions, including disorders of the central nervous system (CNS), with several clinical trials currently underway for stroke and macular degeneration. However, R/NIR irradiation therapy (R/NIR-IT) has not been widely adopted in clinical practice for CNS injury or disease for a number of reasons, which include the following. The mechanism/s of action and implications of penetration have not been thoroughly addressed. The large range of treatment intensities, wavelengths and devices that have been assessed make comparisons difficult, and a consensus paradigm for treatment has not yet emerged. Furthermore, the lack of consistent positive outcomes in randomised controlled trials, perhaps due to sub-optimal treatment regimens, has contributed to scepticism. This review provides a balanced précis of outcomes described in the literature regarding treatment modalities and efficacy of R/NIR-IT for injury and disease in the CNS. We have addressed the important issues of specification of treatment parameters, penetration of R/NIR irradiation to CNS tissues and mechanism/s, and provided the necessary detail to demonstrate the potential of R/NIR-IT for the treatment of retinal degeneration, damage to white matter tracts of the CNS, stroke and Parkinson's disease.

Keywords: irradiation therapy; Parkinson's disease; retinal degeneration; trauma – nervous system.

***Corresponding author: Melinda Fitzgerald,** Experimental and Regenerative Sciences; and School of Animal Biology, The University of Western Australia, Crawley 6009, Australia, e-mail: lindy.fitzgerald@uwa.edu.au

Stuart Hodgetts and Alan R. Harvey: Experimental and Regenerative Sciences; and School of Anatomy, Physiology and Human Biology, The University of Western Australia, Crawley 6009, Australia

Corinna Van Den Heuvel and Robert Vink: School of Medical Sciences, The University of Adelaide, Adelaide 5005, Australia

Riccardo Natoli, Krisztina Valter and Jan Provis: ANU Medical School and John Curtin School of Medical Research, The Australian National University, Acton 0200, Australia

Nathan S. Hart: School of Animal Biology, The University of Western Australia, Crawley 6009, Australia

Sarah A. Dunlop: Experimental and Regenerative Sciences; and School of Animal Biology, The University of Western Australia, Crawley 6009, Australia

Introduction

Irradiation in the red/near-infrared spectrum (R/NIR, 630–1000 nm) was developed as a therapeutic strategy for the treatment of a range of injuries and diseases, following observations of beneficial effects on astronauts in space (Whelan et al., 2001). Therapeutic use of irradiation at these wavelengths is characterised by relatively low energy densities and is referred to as R/NIR-IT. It is distinct from high-energy ablative or thermocoagulatory laser treatments, or light dependent imaging techniques. Improvements following R/NIR-IT have been observed in a wide array of clinical conditions, including wound healing (Yu et al., 1997; Whelan et al., 2001, 2003), oral mucositis (Eells et al., 2004), cardial infarct size (Oron et al., 2001) and renal and hepatic complications during diabetes (Lim et al., 2009, 2010), although clinical efficacy is not always clear cut. Specific to the nervous system, beneficial effects have been reported following retinal degeneration (Natoli et al., 2010; Albarracin and Valter, 2012b), central nervous system (CNS) injury (Byrnes et al., 2005; Fitzgerald et al., 2010), stroke (Lapchak et al., 2007), peripheral nerve damage (Rochkind et al., 2009; Ishiguro et al., 2010) and for restless leg syndrome (Mitchell et al., 2011). However, R/NIR-IT has not been widely adopted in clinical practice for a number of reasons. Firstly, the mechanism/s of action is/are still not clear, and the impact of limited penetration of irradiation is unknown. Secondly, the large range of treatment intensities, wavelengths and devices that have been employed make inter-trial comparisons difficult and a consensus paradigm for treatment of CNS injury or disease has not yet emerged from preclinical studies, yet alone clinical ones. Finally, the lack of a definitive positive effect to date in randomised controlled trials, perhaps due to sub-optimal treatment regimens, has contributed to scepticism.

Here, we provide a synthesis of the literature regarding treatment modalities and efficacy of R/NIR-IT for injury and disease in the CNS. We begin by defining the principles of R/NIR-IT, and emphasise the importance of specifying the parameters of wavelength, intensity, duration of treatment and the nature of the irradiation administered to enable comparison across different studies. R/NIR-IT cannot be effective without adequate penetration to the target tissue, and to this end, we have reviewed information describing penetration of R/NIR irradiation to CNS tissue and included additional calculations to aid interpretation. We briefly address mechanistic elements common to R/NIR-IT therapy across a range of disease and injury conditions and then comprehensively review the literature referring to retinal degeneration, damage to the optic nerve (ON) and visual cortex, spinal cord injury (SCI), traumatic brain injury (TBI), stroke and Parkinson's disease. Further, we provide a detailed tabulated comparison of the studies described, with information detailing the models used, irradiation source, intensity, wavelengths, duration of treatments and outcomes. We conclude by providing an assessment of the potential clinical application of R/NIR-IT for treatment of CNS injuries and disorders.

Parameters of R/NIR-IT

Despite positive results from preclinical research studies and successful clinical trials, as well as the obvious appeal of a relatively cheap and easily administered therapy, R/NIR-IT remains controversial and little used in mainstream medicine (Huang et al., 2011). This is in part due to the lack of a standardised technical approach: a search of the literature reveals a bewildering array of irradiation sources (laser or light-emitting diode), mode of delivery (pulsed or continuous), stimulation wavelength (630, 670, 780, 810, 830, 880 or 904 nm), total dose (i.e., joules of irradiation per unit area), rate of delivery of the irradiation energy [watts per unit area (note: watts=joules×time), also referred to as fluence], duration (length of exposure), timing (pre- or post-insult) and frequency of treatment (Quirk and Whelan, 2011). This is confounded by the fact that dosages are usually specified in energy rather than quantal units; because photon energy varies with wavelength, an equal energy dose at different wavelengths will comprise different numbers of photons, and it is the number of photons interacting with a target photoacceptor that define the actual dose. Although a range of treatment parameters have been trialled, we find few studies where variations in wavelength, total dose and dose rate have been tested in the same model. In short, although the empirical basis of R/NIR-IT for CNS injury and neurodegenerative disease is sound, its clinical application is hampered by uncertainty regarding treatment parameters. As an example of this variability, a comparison of the parameters used in pre-clinical studies of R/NIR-IT for treatment of CNS injury or Parkinson's disease is provided in Table 1.

Penetration of irradiation in the human brain

The extent to which R/NIR irradiation can penetrate the brain is a key determinant of potential efficacy. Here, we describe factors affecting penetration and give some biophysical examples to demonstrate that extremely low irradiation levels are sufficient to affect cells.

Extent of penetration (transmission)

When irradiation strikes biological tissue, it is absorbed, scattered or transmitted. Optimal penetration within biological tissues occurs within a 'therapeutic' or 'optical' window with a wavelength range of 600–1000 nm (Parrish, 1981). The effective penetration depth of a given wavelength of irradiation is dependent upon the optical properties of the tissue, i.e., absorption and scattering (Cheong et al., 1990). Irradiation in the range of 600–1000 nm penetrates tissue because scattering by tissue inhomogeneities is dominant (Profio, 1989). Scattering increases the distance travelled by photons, thus diffusing the propagating irradiation. Absorption occurs predominantly by chromophores such as melanin and haemoglobin at short wavelengths and water and cytochrome c oxidase, a photoacceptor within the mitochondrial electron transport chain, at longer wavelengths (Karu, 1989; Sutherland, 2002).

Detailed characterisation of irradiation distribution in tissues is highly complex and ultimately requires the extrapolation of measurements that, for technical reasons discussed further below, must be made on samples that are considerably thinner than the tissue or organ of interest (Lenz, 1999). Issues such as tissue fixation, the limitations in availability and access to appropriate regions within living tissue as well as limitation of the sensitivity of available equipment used to measure irradiation intensity must all be considered. However, the theory of

Table 1 A summary of R/NIR-IT pre-clinical studies for treatment of neurotrauma and neurodegenerative disease.

Study	*In vitro*: cell type, species	*In vivo*: model, species	Light source	Wave-length	Intensity, density	Treatment duration	Trial length	Key outcomes
Retinal degeneration								
Eells et al. (2003)	NA	Methanol toxicity, rat	LED, Quantum Devices, Barneveld, WI, USA (Warp 10)	670 nm	28 mW/cm^2 4 J/cm^2	144 s, 5, 25, and 50 h post-methanol	2 days	Photoreceptors protected from methanol toxicity (ERG and histology)
Kokkinopoulos et al. (2012)	NA	Ageing, mice	LED, Quantum Devices (Warp 10)	670 nm	40 mW/cm^2 3.6 J/cm^2	5×90 s, every 7 h (35 h total treatment time)	7 days	Shifted mitochondrial potential, reduced inflammation in outer retina
Albarracin et al. (2012)	NA	Retinal light damage (1000 lux), rat	LED, Quantum Devices (Warp 75)	670 nm	60 mW/cm^2 9 J/ cm^2	176 s/day for 5 days, delivered pre- or post-light damage	13 days	Increased protection and survival of cone photoreceptors
Albarracin et al. (2011)	NA	Retinal light damage (1000 lux), rat	LED, Quantum Devices (Warp 75)	670 nm	60 mW/cm^2 9 J/ cm^2	176 s/day for 5 days, delivered pre-light damage	36 days	Increased long-term stability of the retina following acute light damage
Qu et al. (2012)	NA	Retinal light damage (900, 1800, 2700 lux), rat	LED, Quantum Devices (Warp 10)	670 nm	50 mW/cm^2 90 J/ cm^2	1800 s, 3 h before and 0, 24 and 48 h post-light damage	2 days	Protective effect on retinal cells
Natoli et al. (2010)	NA	Retinal light damage (1000 lux), rat	LED, Quantum Devices (Warp 75)	670 nm	60 mW/cm^2 9 J/ cm^2	176 s/day for 5 days, delivered pre-light damage	6 days	Photoreceptor protection, modulation retinal gene expression
Peoples et al. (2012)	NA	Parkinson's (MPTP–100 mg/kg), mice	LED, Quantum Devices (Warp 10)	670 nm	5 mW/cm^2 0.5 J/cm^2	90 s, Delivered T0 and post-insult	56 days	Protection of retina from parkinsonian insult
Albarracin et al. (2012)	NA	Retinal light damage (1000 lux), rat	LED, Quantum Devices (Warp 75)	670 nm	60 mW/cm^2 9 J/ cm^2	176 s	13 days	Increased neuroprotection in retina, possibly via Müller cells
Rojas et al. (2008)	NA	Rotenone inhibition, rat	LEDtronics, Torrance, CA, USA	633 nm	2 mW/cm^2 3.6 J/cm^2	1800 s	16 days	Neuroprotection against rotenone toxicity
Visual system								
Wong-Riley et al. (2001)	Visual cortex neurons, rat	NA	LED, Quantum Devices	670 nm	50 mW/cm^2, 4 J/cm^2	80 s/day	6 days	Increased COX activity
Wong-Riley et al. (2005)	Visual cortex neurons, rat	NA	LED, Quantum Devices	670 nm 728 nm 770 nm 830 nm 880 nm	50 mW/cm^2, 4 J/cm^2	80 s/day, once or twice/day	6 days	670, 830 nm increased ATP and COX with COX inhibitor KCN, but not tetrodotoxin
Liang et al. (2006)	Visual cortex neurons, rat	NA	LED, Quantum Devices	670 nm	50 mW/cm^2, 30 J /cm^2	10 min	28 h	Decreased ROS and apoptosis induced by KCN

(**Table 1** Continued)

Study	*In vitro*: cell type, species	*In vivo*: model, species	Light source	Wave-length	Intensity, density	Treatment duration	Trial length	Key outcomes
Fitzgerald et al. (2010)	NA	Partial ON injury, rat	LED, Quantum Devices (Warp 10)	670 nm	25 mW/cm^2,	30 min/day	8–10 days	Reduced ox stress, limited OPC increases, restored visual function
Assia et al. (1989)	NA	ON crush, rat	He-Ne laser	630 nm	10.5 mW	2 min/day, for 4, 7 or 14 days	14 or 21 days	Increased CAP; if moderate injury, early and continued
SCI								
Byrnes et al. (2005)	NA	SCI dorsal hemi-section T9, rat	Laser, Thor International	810 nm	150 mW, 1589 J/cm^2/ day (at skin)	50 min/day for 14 days (starting 15 min after surgery)	9 weeks	Improved axonal number, regrowth, and some function. Immunomodulation
Wu et al. (2009)	NA	SCI contusion T10, dorsal hemi-section T9, rat	Laser, Thor International (Chesham, UK)	810 nm	150 mW 1589 J/cm^2/ day (at skin)	50 min/day for 14 days (starting immediately after surgery)	3 weeks	Increased axon length and number, and open field (BBB) locomotion in contusion model
Medalha et al. (2010)	NA	SCI complete transection T9–10, rat	Laser, Teralaser (MM Optics Ltd., Sao Carlos, Brazil)	830 nm	30 mW/cm, 250 J/cm^2 (CW)	70 s/day 3×/wk at two points on hindlimb, (not on spinal cord)	4 weeks	No improvements. Note: treatment not applied directly to spinal cord
TBI								
Oron et al. (2007)	NA	TBI, acute cortical impactor, 94 g, mice	Laser, Photothera, Inc. (Carlsbad, CA, USA)	808 nm	10 and 20 m/W/cm^2 (1.2–2.4 J/cm^2)	2 min (at 4 h post-trauma)	4 weeks	Improved neurobehavioural function (NSS) and reduced lesion size
Moriera et al. (2009)	NA	TBI, focal cryoinjury, rat	Laser Teralaser (MM Optics Ltd)	660 nm 780 nm	40 mW 3–5 J/cm^2/point (CW)	Immediately and 3 h after TBI	24 h	Immunomodulation of TNF-α, IL10, IL1β cytokine responses
McCarthy et al. (2010)	NA	Uninjured brain, rat	Laser ND	808 nm	2230 mW/cm^2 268 J/cm^2 or 10 mW/cm^2, 1.2 J/cm^2	5 min on day 1, or on each of days 1, 3, 5	1 year	Single and multiple doses safe
Ando et al. (2011)	NA	TBI, acute, AMS 201 impactor, mice	Laser, DioDent Micro 810, (Hoya Conbio, Fremont, CA, USA)	810 nm	50 mW/cm^2, 36 J/cm^2, 10-Hz CW; 100-Hz PW	12 min at 4 h post-TBI	4 weeks	Improved NSS, reduced lesion volume, anti-depressant. 10-Hz PW better effect
Naeser et al. (2011)	NA	Chronic TBI, human (females, 59 years, 52 years)	Laser, 2 devices, ND	633 nm, 870 nm (CW)	25.8 mW/cm^2 or 22.2 mW/cm^2	310–774 s/week 7 months, then 3 weeks/month or 7–10 min/day	7 years 4 months	Improved executive function, reduced post-traumatic stress
Nawashiro et al. (2012)	NA	TBI, human (male, 40 years)	Laser, Sun-Mechatronics (Tokyo, Japan)	830–870 nm	299 mW 11.4 mW/cm^2, 20.5 J/cm^2	2×30 min/day, applied 5 mm from skin	73 days	Some neurological improvement, increased cerebral blood flow

(**Table 1** Continued)

Study	*In vitro*: cell type, species	*In vivo*: model, species	Light source	Wave-length	Intensity, density	Treatment duration	Trial length	Key outcomes
Khuman et al. (2012)	NA	TBI, cortical piston, 3 mm wide, 6 m/s, 100 ms, depth 0.6 mm, mice	Laser, Thor International	800 nm	Craniotomy: 250–500 mW/cm^2 30–210 J/cm^2 Transcranial: 500 mW/cm^2	Craniotomy: 2–7 min, 60–80 min post-TBI Transcranial: 2 min, 60–80 min or 4 h post-TBI	1 week	Improved cognition inhibition of microglia. No effect on motor function, oedema, nitrosative stress or lesion volume
Wu et al. (2012)	NA	TBI, acute impactor, mice	Laser, Diomed, Inc, or V-Raser, (Con-Bio)	665 nm 730 nm 810 nm 980 nm	36 J/cm^2 (CW)	1×4 min, at 4 h post-injury	4 weeks	Improved NSS, reduced brain 'deficits' with 665 nm and 810 nm
Quirk et al. (2012)	NA	TBI, cortical impactor, 6 mm/s, 3 mm, rat	LED, Quantum Devices	670 nm	50 mW/cm^2, 15 J/cm^2	2×5 min/day for 72 h or 10 days (top of head, 0.5 cm from scalp)	10 days	Improved NSS, decreased Bax, increased Bcl2
Oron et al. (2012)	NA	TBI, cortical impactor, 94 g mice	Laser, Photothera Inc.,	808 nm	10 m/W/cm^2 1.2 J/cm^2	2 min (4, 6, or 8 h post-trauma), PW 100 or 600 Hz	56 days	Improved NSS reduced lesion size
Stroke								
Lapchak et al. (2004)		RSCEM, rabbit	Acculaser PhotoThera, Inc.	808 nm	25 mW/cm^2 or 7.5 mW/cm^2 (CW)	2 or 10 mins at 1 or 24 h post-insult	Up to 21 days	Improved behavioural performance
DeToboada et al. (2006)		MCAO, rats	Ga-Al-As diode laser, PhotoThera	808 nm	7.5 mW/cm^2	2 min	Up to 28 days	Reduced neurological deficits
Oron et al. (2006)		MCAO, rats	Ga-Al-As diode laser, PhotoThera, Inc	808 nm	7.5 mW/cm^2 (CW and/or PW)	2 min at 4 or 24 h post-stroke	Up to 21 days	Improved neurological outcome with 24 h. No improvement in stroke volume
Lapchak et al. (2007)		RSCEM, rabbits	Acculaser, PhotoThera, Inc.	808 nm	7.5 mW/cm^2 (CW or PW)	2 min at 6 or 12 h post-embolisation	48 h	Significant improvement with PW at 6 h
Lapchak et al., 2008. Stroke. 39 p. 307303078		RSCEM, rabbit	Acculaser, PhotoThera, Inc.	808 nm	10 mW/cm^2	2 min at 90 min post-embolisation	24 h	Safe with tPA, haemorrhage and volume unaffected
Lapchak and DeTaboada 2010. Brain Res. 1306 p. 100–105		RSCEM, rabbit	Ga-Al-As diode laser, PhotoThera, Inc.	808 nm	7.5 mW/cm^2 (CW) 37.5 mW/cm^2 (PW1,100 Hz) 262.2 mW/cm^2 (PW2,100 Hz)	5 min post-embolization CW 2 min PW1 2 min, 3.5 mJ/pulse, PW2 2 min, 24.5 mJ/pulse	3 h	Increased cortical ATP content

(**Table 1** Continued)

Study	*In vitro*: cell type, species	*In vivo*: model, species	Light source	Wave-length	Intensity, density	Treatment duration	Trial length	Key outcomes
PD								
Ying et al. (2008)	Cortical neurons; rotenone and MPTP, rat	NA	LED, Quantum Devices	670 nm	50 mW/cm^2; 4 J/cm^2	80 s 2×/day	2–4 days	Reduced apoptosis and increased ATP content
Liang et al. (2008)	Neurons; rotenone and MPTP, rat	NA	LED, Quantum Devices	670 nm	50 mW/cm^2; 4 J/cm^2	80 s 2×/day	3–5 days	Increased ATP content, decreased neuronal apoptosis, reduced ROS and NO
Shaw et al. (2010)	NA	Acute PD, 50–100 mg/kg ip MPTP over 30 h, mice	LED, Quantum Devices	670 nm	40 mW/cm^2; 5.3 J/cm^2	90 s; 4×over 30 h	30 h	Increased number of surviving dopaminergic cells in substantia nigra
Peoples et al. (2012)	NA	Acute and chronic PD; 10×20 mg/kg ip over 5 weeks, mice	LED, Quantum Devices	670 nm	40 mW/cm^2; 5 J/cm^2	90 s; 10×	3–8 weeks	Increased number of surviving dopaminergic cells in substantia nigra
Other								
Yan et al. (2011)	NA	Pain, rat	Irradia™ (Stockholm, Sweden)	650 nm 808 nm	35 mW 450 mW	30 s to each of 4 points		Decreased SSEP and CMAP amplitudes indicated potential pain relief

Abbreviations for laser type are combinations of the following elements: gallium (Ga), aluminium (Al) or arsenide (As). Abbreviations: BBB, Basso, Beattie and Bresnahan; COX, cytochrome *c* oxidase; CW, continuous wave; PW, pulsed wave; NA, not appropriate; ND, not described; NSS, neurological severity score; SCI, spinal cord injury; ON, optic nerve; TBI, traumatic brain injury; OPCs, oligodendrocyte precursor cells; CAP, compound action potentials; s, seconds; min, minutes; h, hours; RSCEM, small clot embolic stroke model; MCAO, middle cerebral artery occlusion; SSEP, somatosensory evoked potential; CMAP, comprehensive muscular activity profile.

irradiation transmission through highly scattering media is well established, and theoretical approximations commonly used to derive optical penetration depths (delta, δ) closely follow empirical measurements (Stolik et al., 2000). δ is the tissue thickness that causes irradiation to be attenuated to 37% of its initial value (Muller and Wilson, 1986), i.e., not the maximum distance that irradiation will penetrate a tissue sample.

By way of example, we have used a diffusion model (Svaasand and Ellingsen, 1983) to calculate irradiation penetration to the centre of the human brain. We assumed brain dimensions of W140 mm×L167 mm×H93 mm and a surface irradiance of 60 mW/cm^2 (e.g., the FDA-approved Vet 75 device at 670 nm, Quantum Devices Inc., Barneveld, WI, USA) and that the head would be illuminated from the top, with the irradiation source positioned above the shaved scalp. We also assumed that the optical penetration depth of the skull is similar to that of the overlying skin and the brain (Firbank et al., 1993), which suggests that all these tissues (and any other sub-cranial tissues and fluids) pass irradiation at any particular wavelength in a similar fashion, thereby simplifying calculations.

Calculated fluence rates just inside the cranium (assumed to be ~7 mm thick with a 3-mm-thick skin covering to give a total thickness of 10 mm) were calculated to be 2.5 mW/cm^2 for 670-nm irradiation (δ=2.4 mm^2) and 13 mW/cm^2 for 1064 nm (δ=4.0 mm^2). Note that these two wavelengths are chosen as they are commonly used in R/NIR-IT; 670-nm irradiation is thought to change the oxidation reduction state of cytochrome *c* oxidase (see below), and 1064 nm irradiation is transmitted better through biological tissues (Karu, 1989; Sutherland, 2002). Indeed, greater transmission of irradiation at longer wavelengths has been shown previously for a total human scalp and skull thicknesses of up to 13 mm (Wan et al., 1981). By contrast, and due to the exponential relationship between irradiation penetration and tissue thickness, fluence rates in the centre of the brain (10-mm skull and skin +46-mm brain=56 mm) were calculated to be very much lower at 1.2×10^{-11} W/cm^2 for 670-nm irradiation and 1.4×10^{-7} W/cm^2 at 1060 nm. The considerably greater (10^4) value for the penetration of longer wavelength irradiation calculated to reach the centre of the brain is in agreement with other studies comparing tissue penetration for a variety of wavelengths (Eichler et al., 1977; Lenz, 1999; Neupane et al., 2010).

To our knowledge, only two studies have directly measured the penetration of R/NIR irradiation in intact animals. Spectrophotometric and power transmission analyses in rats revealed that 6% (9 mW) of transcutaneous 810-nm laser irradiation (power output 150 mW) was transmitted from the dorsal surface of the skin to the ventral side of the spinal cord (Byrnes et al., 2005). Similarly, a study in rats showed that when irradiation was directed at the dorsal surface of the head (total R/NIR irradiance 252 W/m^2, 550–750 nm), 0.7% (1.75 W/m^2) reached the ventral surface of the optic nerve, and 0.1% (0.3 W/m^2) reached the ventral surface of the braincase (Fitzgerald et al., 2010).

Factors affecting penetration

A number of factors affect R/NIR irradiation penetration of tissue. Haemoglobin and water are major chromophores, i.e., they absorb irradiation (Sterenborg et al., 1989), thus the extent of R/NIR irradiation penetration will presumably vary according to vascularisation and fluid balance. Although the absorption coefficient of water is low in the visible region, it is significant for R/NIR irradiation, and the large volume fraction of water in biological tissue, together with haemoglobin, contributes significantly to absorption (Ankri et al., 2010). Indeed, in *ex vivo* human kidney and liver tissue, δ is greater for slices containing blood compared to those in which the blood has been removed (Eichler et al., 1977). Furthermore, δ values obtained from freshly resected human brain tissue were greater in malignant brain tumours that were more vascularised, compared to normal brain tissue (Muller and Wilson, 1986). Both vascularisation and water balance are profoundly influenced in neurodegenerative disease, such as Alzheimer's disease (AD) and Parkinson's disease (PD) as well as stroke. For example, β-amyloid promotes angiogenesis and blood-brain barrier permeability in Tg2576 AD mice (Biron et al., 2011) and in humans with cerebral amyloid angiopathy (Hartz et al., 2012), whereas integrin αvβ3, a marker for angiogenesis, is increased in human PD brains (Desai Bradaric et al., 2012). Aquaporin expression, indicative of changed water transport, is enhanced in AD brains (Moftakhar et al., 2010), and blood-brain barrier permeability is increased following ischaemic stroke (Topakian et al., 2010; Hom et al., 2011). Not unexpectedly, vascular damage is also extensive following TBI, both acutely (Iwamura et al., 2012) and with evidence for long-term remodelling (Rodríguez-Baeza et al., 2003). Reactive gliosis is a further contributor to changes in cellular architecture that may modulate penetration of R/NIR-IT (Sykova and Vargova, 2008). However, in these pathological conditions, it is unknown whether the increased blood supply and changed water balance would act as a more prominent substrate for the absorbance of R/NIR irradiation during therapy.

Oxygenation also influences penetration of irradiation. Compared to room air (21% O_2, 0.039% CO_2), carbogen breathing (95% O_2, 5% CO_2) in mice increased the penetration of R/NIR irradiation and improved the outcome of photodynamic therapy for tumour ablation (Mitra and Foster, 2004). Conversely, in situations where hypoxia occurs, such as after traumatic and hypoxia-ischaemia-induced brain injury (Oddo et al., 2011; Howards et al., 2012), any therapeutic use of R/NIR irradiation may require higher intensity irradiation to offset reduced tissue penetration. Myelination is also thought to influence penetration of irradiation, with greater δ values in grey compared to white matter (Lenz, 1999). A study of bovine brain showed that irradiation penetration was greatest when irradiation was oriented parallel to white matter tracts (Hebeda et al., 1994), a finding that explains the increased incidence of lesions within the corpus callosum after photodynamic therapy in normal mouse brain (Sandeman et al., 1986).

Extremely low irradiation levels can affect cells

The foregoing calculations suggest that fluence/fluence rates at limited depths into the skull and brain are quite significant. A 20-min exposure to a 60-mW/cm^2 670-nm LED array (e.g., Vet 75) positioned above the head would give a total fluence (irradiation dose) of ~3 J/cm^2 at a fluence (dose) rate of 2.5 mW/cm^2 to the surface of the brain just under the cranium. This is within known effective dose/rate ranges (cf. Huang et al., 2011). Whether or not the extremely low level of irradiation reaching the centre of the brain is 'effective' for irradiation therapy of CNS injury or disease remains to be determined.

Nevertheless, very low levels of irradiation can trigger significant biophysical phenomena. For example, at the threshold of useful vision, such as when we are able to detect the edges of large objects at night under a moonless, overcast sky, the ambient light intensity is such that (on average) each rod photoreceptor in the retina only absorbs a single photon of light or irradiation every 84 min. Even at dawn, when there is enough light to see clearly and objects appear coloured because cones are also active, rods are only capturing (on average) one photon every 5 s (Rodieck, 1998). However, because the retina contains approximately 90 million rods (Curcio et al., 1990), we are able to use this limited information to form an image and not just detect light. To put this into context, the intensity of the dimmest extended light source that can be seen by a human corresponds to a fluence rate of 1.5×10^{-14} W/cm^2, i.e., well below that reaching the centre of the brain in our modelled scenario. Although the exact mechanism of R/NIR-IT remains to be elucidated, it is clear that the low levels of irradiation encountered are more than capable of triggering cellular signalling and probably sufficient to drive metabolic events.

Potential mechanisms of efficacy of R/NIR-IT

Several recent reviews have summarised existing knowledge regarding the potential mechanisms by which R/NIR-IT exerts its effects (Hashmi et al., 2010; Chung et al., 2012), and we therefore address this subject relatively briefly. Cytochrome *c* oxidase is proposed to act as a photoacceptor for irradiation at these wavelengths, with absorption spectra matching efficacious wavelengths and irradiation leading to changes in the oxidation reduction state of the enzyme (Karu, 1999; Karu and Kolyakov, 2005; Karu et al., 2005, 2008). Increases in cytochrome *c* oxidase activity with R/NIR-IT are associated with increases in ATP content in treated tissues, indicating increased flux through the electron transport chain (Wong-Riley et al., 2005; Lapchak and De Taboada, 2010). Although it has been proposed that these changes are associated with increased reactive oxygen species (ROS) and resultant downstream signalling (Hashmi et al., 2010; Chung et al., 2012), it is important to note that studies linking various facets of oxidative metabolism to ROS and reactive nitrogen species (RNS) production have been contradictory, largely conducted *in vitro*, and highly dependent on the timing of the experimental observations and the conditions employed (Tretter et al., 2007; Peng and Jou, 2010). Indeed, it is possible that an increased flux of electrons through the electron transport chain may maintain mitochondrial membrane potential, reduce passage through the reverse electron transport chain, alter cAMP release and increase ATP synthesis, all of which may result in reduced leakage of free radical intermediates (Camello-Almaraz et al., 2006; Rojas et al., 2008; Kowaltowski et al., 2009). Although direct deductions concerning the sequence of effects in mitochondria with R/RIR-IT are problematic with the existing information available to us, R/NIR-IT has been shown to improve indices of mitochondrial function following damage to the CNS in a range of model systems, many of which have been associated with improvements in function (Eells et al., 2004; Rojas et al., 2008). Further descriptions of reported effects of R/NIR-IT on cytochrome *c* oxidase and mitochondrial function in

specific CNS injuries and disease states are provided in the sections below.

Although a significant proportion of the available data on the mechanism of R/NIR-IT point towards cytochrome *c* oxidase as a primary photoacceptor, this does not preclude other potential modes of action. The chromophores melanin and haemoglobin may also play roles (Karu, 1989; Sutherland, 2002; Peoples et al., 2012a). Nitric oxide released from cytochrome *c* oxidase may lead to downstream vasodilatation (Mason et al., 2006; Ball et al., 2011) and signal transduction, potentially also contributing to functional improvements. R/NIR-IT has been shown to modulate gene expression (Natoli et al., 2010), reduce apoptosis (Wong-Riley et al., 2005; Liang et al., 2008), alter cytokine release and modulate immune responses (Moreira et al., 2009; Albarracin and Valter, 2012a; Kokkinopoulos et al., 2012; personal observation), and these outcomes may be upstream, downstream or independent of the modulation of cytochrome *c* oxidase activity.

R/NIR-IT for treatment of retinal degeneration

The studies discussed here employ R/NIR LED devices to deliver a therapeutic dose to the retina in an experimental setting or to monitor effects on the ageing retina. The high metabolic activity of photoreceptors renders the retina highly susceptible to oxidative damage (Winkler, 1981; Yu et al., 1999). Oxidative damage to photoreceptors has been implicated in many forms of retinal degeneration, including age-related macular degeneration (AMD) (Age-Related Eye Disease Study Group, 2001), retinitis pigmentosa (Shen et al., 2005), retinopathy of prematurity (Tsukahara et al., 2004) and in the later stages of all photoreceptor degenerations regardless of the initiating event (Stone et al., 1999). Arguably, amelioration of oxidative damage is the key to long-term survivability of the retina. Several laboratories have now used R/NIR irradiation to attenuate experimentally induced retinal degeneration, including models of AMD (Natoli et al., 2010; Qu et al., 2010; Albarracin et al., 2011; Albarracin and Valter, 2012a,b), Parkinson's-related retinopathy (Peoples et al., 2012a) and methanol (Eells et al., 2003) and rotenone (Rojas et al., 2008) toxicity, with beneficial effects in normal ageing (Kokkinopoulos et al., 2012). None of these studies report any adverse effects of 670-nm irradiation on the retina.

Methanol is a potent mitochondrial toxin that inhibits cytochrome *c* oxidase activity (Seme et al., 1999). Eells et al. (2003) reported the first direct link between the mode of action of R/NIR irradiation in retinoprotection using a methanol toxicity model. They found that mitochondrial damage in photoreceptors caused by methonal toxicity was reduced by exposure to 670-nm irradiation. Mitochondria retained their normal structure in animals intoxicated with methanol and treated with 670-nm irradiation. Rod and cone response amplitudes (electroretinogram) were reduced up to 75% by methanol toxicity; however, when combined with 4-J/cm^2 treatments of 670-nm irradiation at 5, 25 and 50 h of methanol intoxication, the response amplitudes were reduced by only ~33%. The authors concluded that because formic acid (derived from the breakdown of methanol) acts directly to inhibit cytochrome *c* oxidase, a key enzyme in mitochondrial metabolism, 670-nm irradiation appeared to be directly modulating enzyme activity to reduce this toxic effect and promote mitochondrial function. In an *in vivo* study in rat retina, 633-nm irradiation was protective against rotenone, a potent inhibitor of mitochondrial function, providing a further direct link between these organelles and R/NIR irradiation (Rojas et al., 2008). This link has also been noted in experiments using the mitochondrial dye JC-9, in which there is a shift in mitochondrial membrane potential in retinal pigment epithelial cells in direct response to 670-nm irradiation (Kokkinopoulos et al., 2012).

Oxidative damage generated by excessive photo-oxidation of rod outer segments is thought to be the initiating event in light-induced retinal damage (LD) (Demontis et al., 2002). However, it has also been shown in LD that retinal degeneration continues long after removal of the damaging stimulus (white light) (Rutar et al., 2010). This progressive degeneration has been used to model the factors contributing to the expansion of the degenerative area, as occurs in AMD, and appears to be largely mediated by inflammation (Hollyfield et al., 2008). Microarray analysis (Natoli et al., 2010) shows that the expression of genes in pathways involved in inflammation, apoptosis and metabolism are down-regulated in LD retinas treated with 670-nm irradiation. One of the most highly modulated genes identified in that study is *Ccl2* - a potent chemokine involved in the recruitment of macrophages to sites of tissue injury. *Ccl2* has become a gene of interest from investigations in models of AMD, and this chemokine family is now implicated in its pathogenesis (Rutar et al., 2011).

Several studies have found that 670-nm irradiation is protective against retinal degeneration in LD, citing histological, functional and molecular evidence (Natoli et al., 2010; Qu et al., 2010; Albarracin et al., 2011; Albarracin and Valter, 2012a). Treatment with 670-nm irradiation before, during or after exposure to damaging white

light attenuates retinal degeneration (Qu et al., 2010; Albarracin et al., 2011), protects photoreceptor function and reduces the expression of stress markers in the retina, as well as microglial and macrophage invasion (Albarracin and Valter, 2012a). Although treatment prior to exposure to bright light is most effective, animals treated with 670-nm irradiation *after* light damage recover photoreceptor function by 1 month post-exposure (Albarracin et al., 2011). LD induces upregulation of a number of markers of oxidative stress, and these are downregulated by 670-nm irradiation (Natoli et al., 2010). Two independent studies report downregulation of the pro-inflammatory cytokine TNF-alpha following treatment with 670-nm irradiation. In the first, quantitative polymerase chain reaction (PCR) was used to show a reduction in TNF alpha levels in the LD retina pre-treated with 670-nm irradiation (Albarracin and Valter, 2012a). In the second, it was shown that treatment of aged mice with 670-nm irradiation reduces TNF-alpha immunoreactivity, as well as recruitment of IBA1-positive macrophages to the outer retina, and C3b and C3d immunoreactivity in Bruch's membrane (Kokkinopoulos et al., 2012), all indicating downregulation of inflammatory responses. In addition, components of the 'classical' pathway of complement activation, as well as C3 are downregulated by pretreatment with 670-nm irradiation in the LD model (unpublished observation).

Collectively, these studies provide strong evidence that 670-nm irradiation gives significant protection to the retina, and some studies indicate that cytochrome *c* oxidase is the most likely photoacceptor. The observed effects can be explained by a theoretical model of 670-nm IT promoting effective mitochondrial function, resulting in a reduction of free radical production and oxidative damage. The downstream effects appear to be a downregulation of inflammatory processes. The LD model specifically, and the retina in general, are ideal models to explore the mechanisms, potential and limitations of R/NIR-IT due to the relative ease of inducing damage and the known interactions between oxidative stress and inflammatory pathways, combined with accessibility for treatment.

R/NIR-IT for treatment of damage to the optic nerve and visual cortex

Evidence supporting the role of cytochrome *c* oxidase as a key photoacceptor for irradiation in the R/NIR spectrum has also been generated in studies of neurons from the visual cortex. *In vitro* experiments assessing the effects of inhibitors on neurons from the post-natal rat visual cortex demonstrate that potassium cyanide (KCN) inactivation of cytochrome *c* oxidase (by 100 μM KCN or less) is reversed by treatment with 670-nm irradiation delivered by LED array once or twice daily for 100 s (energy density=4 J/cm^2, power density=50 mW/cm^2) (Wong-Riley et al., 2001, 2005). Effects with twice-daily treatments are more pronounced for neurons lightly reactive for cytochrome c oxidase activity (Wong-Riley et al., 2005). Longer pretreatments with 670-nm irradiation (10 min prior to KCN exposure, equivalent to 30 J/cm^2) significantly reduce nuclear condensation (Wong-Riley et al., 2005) attributed to reduced apoptosis and associated with reduced oxidative stress (Liang et al., 2006). The effects of a range of wavelengths of irradiation, delivered via LED array in the R/NIR spectrum, on cytochrome *c* oxidase activity following blockade of voltage-dependent sodium channels with tetrodotoxin have also been compared in visual cortical neurons. Irradiation of 670 nm and 830 nm (energy density=4 J/cm^2, power density=50 mW/cm^2) restores cytochrome *c* oxidase activity and ATP content, whereas 728-nm, 770-nm and 880-nm irradiation are less effective (Wong-Riley et al., 2005). Effective wavelengths correlate positively with the known absorption spectra of oxidised cytochrome *c* oxidase (Carter and Palmer, 1982; Karu, 1999; Wong-Riley et al., 2005; Karu et al., 2008).

Cytochrome *c* oxidase activity in retinal ganglion cell (RGC) somata has been linked to the survival of these cells following complete ON transection (von Bussmann et al., 1993). Although cytochrome *c* oxidase activity is higher in unmyelinated than myelinated regions of the human ON (Balaratnasingam et al., 2009), we have recently demonstrated *in vivo* that cytochrome *c* oxidase activity is increased in the ON following partial ON transection and 670-nm irradiation treatment (LED, 30 min/day, 25 mW/cm^2) and that activity is colocalised with oligodendrocytes, at least in the short term (unpublished observation). Increased cytochrome *c* oxidase activity in the ON is associated with reduced oxidative stress reactive species, both in nerve homogenates and, more specifically, in astrocytes (Fitzgerald et al., 2010) and oligodendrocytes (unpublished observation). Irradiation with 670-nm (LED) treatment also decreases proliferation of oligodendrocyte progenitor cells (Fitzgerald et al., 2010) and reduces paranode elongation in injured ONs vulnerable to secondary degeneration, which is associated with later preservation of RGC numbers and restoration of visual function (Fitzgerald et al., 2010; unpublished observation). Similarly, daily treatments of rat ON crush injuries with 630-nm irradiation (delivered by He-Ne laser, 10.5 mW) for 2 weeks significantly increases compound action potentials in the ON *ex vivo* and postpones degeneration

(Assia et al., 1989). For positive effects, treatment needs to be rapidly initiated (< 5 h after injury), maintained and used on moderately rather than severely injured nerves (Assia et al., 1989).

R/NIR-IT for treatment of traumatic brain injury (TBI)

Neuropathological consequences of TBI include disruption in axonal transport leading to axonal swelling followed by secondary disconnection, extensive demyelination and Wallerian degeneration (Brambilla et al., 2006; Johnson et al., 2012; Tang-Schomer et al., 2012). Alterations to mitochondria (including membrane permeability) influence axonal integrity as well as ionic imbalance, oxidative stress and lipid peroxidation (associated with both mitochondrial dysfunction and cytoskeletal degradation), which play a central role post-injury in both axonal degeneration and dysfunction of viable and intact axons (Buki et al., 1999; Maxwell et al., 2003; Johnson et al., 2012). Of the ten TBI studies presented here describing R/NIR-IT in mice (Oron et al., 2007, 2012; Ando et al., 2011; Khuman et al., 2012; Wu et al., 2012), rats (Moreira et al., 2009; Quirk et al., 2012) and humans (Naeser et al., 2011; Nawashiro et al., 2012), eight reported effects in acute (short-term) or sub-acute, and only one in chronic (long-term) (Naeser et al., 2011) contusive TBI. Of these ten studies, nine used lasers to deliver the R/NIR-IT. A further study using laser-delivered R/NIR-IT (McCarthy et al., 2010) provides evidence that treatment with an 808-nm wavelength in the *uninjured* rat brain is safe at single and multiple doses (Table 1), with no treatment-related lesions, neoplasia or other toxicological abnormalities for up to 1 year after injury (McCarthy et al., 2010). Most rodent model studies report statistically significant improvements in outcomes, including neurological severity scores (NSS), evidence of increased axonal numbers and distance of re-growth, reduced lesion size, modulation of apoptotic and inflammatory responses and pronounced anti-depressant effects.

Treatment of TBI generated by a cortical impactor device in mice with an 808-nm Gs-As (gallium-arsenide) diode laser (10 and 20 mW/cm^2, 1.2–2.4 J/cm^2) for 2 min (at 4 h post-trauma) results in no significant improvements in NSS up to 48 h after treatment. However, from days 5–28, NSS are reduced by about 27% in irradiated mice, which also have smaller lesion sizes compared to controls (1.4% vs. 12.1%) (Oron et al., 2007). Continuous wave (CW) GaAIAs (gallium-aluminium-arsenide) 780-nm or InGaAIP 660-nm low level laser irradiation following a 40-s cryoinjury to the brain results in immunomodulation of TNF-α, IL10 and IL1β cytokine responses following R/NIR-IT (Moreira et al., 2009), although no functional recovery experiments were performed in this study. Neuroprotective effects are also seen with 808-nm GaAIAs laser treatment at 50 mW/cm^2 (CW at 10 Hz and pulsed wave (PW) at 100 Hz) for 12 min at 4 h, following contusive TBI in mice. These neuroprotective effects, which are more pronounced after 10 Hz PW frequency treatment than 100 Hz CW, include improved behavioural recovery (NSS), reduced brain lesion volume and a pronounced anti-depressant effect at up to 4 weeks post-TBI (Ando et al., 2011). A 2–7 min exposure to an 800-nm GaAIAs laser treatment at a variety of doses (250, 500 or 1000 mW/cm^2), at 60–80 min or 4 h after contusive TBI in mice results in no effects on post-injury motor function (days 1–7), brain oedema (24 h), nitrosative stress (24 h) or lesion volume (14 days); however, there are improved cognitive outcomes and inhibition of microglia activation (Khuman et al., 2012).

Four weeks after a single 4-min exposure at 4 h post-contusive TBI (36 J/cm^2 CW, 665 nm, 730 nm, 810 nm or 980 nm) in mice, there is improved behavioural recovery (NSS) and reduced brain 'deficits', but only in 665-nm- and 810-nm-treated animals (Wu et al., 2012). Treatment of rats with 670-nm irradiation (at 50 mW/cm^2, 15 J/cm^2) for 2×5 min per day for 72 h or 10 days, post contusive TBI, results in functional (NSS) and morphological improvements, including decreased pro-apoptotic Bax expression and increased anti-apoptotic Bcl2 expression (Quirk et al., 2012). Finally, Oron et al. (2012), using an 808-nm GaAIAs laser to deliver 10 mW/cm^2 (1.2 J/cm^2) treatment (either CW or PW, at 100 or 600 Hz) for 2 min (at either 4, 6, or 8 h post-trauma) following contusive TBI in mice, show improved neurobehavioural function (NSS) and an overall reduction in lesion size at 56 days. It has been proposed, based mainly on *in vitro* cortical neuron models (see above), that R/NIR-IT benefits recovery from TBI by inhibiting apoptosis while increasing mitochondrial activity, transcriptional activation, angiogenesis and neurogenesis (Chung et al., 2012; Huang et al., 2012).

In humans, R/NIR-IT using 830–870-nm irradiation has been tested in a 40-year-old male (2×30-min treatments per day for 73 days, applied 5 mm from the skin at 11.4 mW/cm^2, 20.5 J/cm^2 (Nawashiro et al., 2012) and in two females, aged 52 and 59. In the latter case, R/NIR-IT using 633 or 870 nm was administered either (i) as treatments of 5-min duration and 10 s per area treated for 7 months, then 3 weeks per month at 25.8 mW/cm^2 (CW) or (ii) daily for 7-min duration per area for 1 month, increasing by 1 min per area for each successive month at 22.2 mW/cm^2 (CW)

for treatment durations ranging from 3 months to 7 years (Naeser et al., 2011). In both studies, improved neurological outcomes (including executive function and memory) were reported, as well as reduced post-traumatic stress disorder and improved cerebral blood flow in chronic TBI (Naeser et al., 2011; Nawashiro et al., 2012). Patients were able to eventually self-treat in the home, but benefits are reduced if the daily/weekly treatment frequency is not maintained.

R/NIR-IT for treatment of spinal cord injury (SCI)

Pathological changes following acute SCI are characterised by focal injury (Sekhon and Fehlings, 2001) followed by an expanding wave of secondary degeneration and cell death (Nashmi and Fehlings, 2001; Park et al., 2004; Baptiste and Fehlings, 2006) that is associated with oxidative damage (Liu et al., 1997; Profyris et al., 2004; Keane et al., 2006). Of the three SCI studies presented here, all describe R/NIR-IT using lasers in rats (Byrnes et al., 2005; Wu et al., 2009; Medalha et al., 2010). To date, no R/NIR irradiation treatments have been reported in humans with SCI. In all rat studies, treatment began either immediately (Wu et al., 2009; Medalha et al., 2010) or within 15 min (Byrnes et al., 2005) after SCI, which involved either dorsal hemisection at T9–T10 alone (Byrnes et al., 2005), dorsal hemisection at T9 or moderate contusion at T9–T10 (10 g dropped from 12.5 mm, NYU impactor) (Wu et al., 2009) or complete transection (Medalha et al., 2010) (Table 1).

R/NIR-IT using 810-nm irradiation (2-week treatment at 1589 J/cm^2/day) results in an increased axonal number and distance of regrowth, as well as immunomodulation and some aspects of functional recovery improvement as measured by ladder footfall, run time and hindlimb paw placement—but no open field [Basso, Beattie and Bresnahan (BBB)] assessment (Byrnes et al., 2005). Similarly, 810-nm irradiation treatment (daily for 14 days at 2997 s per day, 1589 J/cm^2/day) results in increased axon length and number in both dorsal hemisection and moderate contusion SCI models (Wu et al., 2009). In the contusion model, improved open field (BBB) locomotion scores were reported with R/NIR-treated rats, reaching BBB scores of 12–13 at 3 weeks after injury (i.e., frequent to consistent weight-supported plantar steps with frequent coordination between forelimbs and hindlimbs) compared to non-treated rats, which reach BBB scores of 9–10 (i.e., occasional weight-supported plantar steps but with no coordination) (Wu et al., 2009). More recently, 830-nm-treated rats showed a 'trend' toward improved hindlimb diaphysis, but no biomechanical or densitometric improvements following complete spinal cord transection (Medalha et al., 2010). No behavioural (locomotory) improvements as measured by BBB scoring were observed in any treatment group. It is of note, however, that the 830-nm treatment was not actually applied directly to the spinal cord in this study, rather at two points on the hindlimb (Medalha et al., 2010).

R/NIR-IT for treatment of stroke

Although at least 1000 agents have been shown to be efficacious in preclinical ischaemic stroke evaluation, to date only intravenous tissue plasminogen activator (tPA) has been approved for clinical use in treating acute ischaemic stroke (Segura et al., 2008). However, the majority of stroke patients either do not meet the strict criteria for treatment with tPA or fail to receive adequate reperfusion. Therefore, additional new therapies are needed. Recently, non-invasive laser therapy has been applied to acute ischaemic stroke patients with positive results; specifically, R/NIR irradiation is applied to the scalp within 24 h of the onset of stroke symptoms. The principle objective of this section is to evaluate the literature regarding R/NIR-IT delivered by laser in experimental stroke models and recent clinical trials, demonstrating that the most advanced application for R/NIR-IT to date is in ischaemic stroke with promising pre-clinical and clinical results. This subject has recently been comprehensively reviewed by Lapchak (2012); as such, details are provided for purposes of comparison with other CNS injury and disease states.

Pre-clinical rabbit studies

Positive findings of the efficacy of R/NIR-IT in reducing lesion volume in myocardial infarction (Ad and Oron, 2001) prompted an investigation into whether a similar procedure would reduce stroke-related behavioural deficits due to the similarities between myocardial and cerebral ischaemia. A small clot embolic stroke model (RSCEM) was used in rabbits treated with transcranial R/NIR-IT, employing CW irradiation at high power densities (25 mW/cm^2) for 10 min. This initial exploratory study found that laser treatment significantly improves behavioural rating scores (reviewed in Lapchak et al., 2012), remaining effective when initiated within 6 h post-occlusion (Lapchak

et al., 2002). PW R/NIR-IT was more beneficial than CW (Lapchak et al., 2007), which is attributed to increased penetration of photons through the brain using the pulsed peaks (Lapchak, 2012).

It is well established that the core ischaemic area following occlusion of a major cerebral artery experiences rapid loss of ATP and energy production with widespread neuronal depolarisation (Streeter et al., 2004). Embolisation-induced decreases of cortical ATP are attenuated by subsequent treatment with either CW R/NIR-IT or PW R/NIR-IT, although PW R/NIR-IT is more effective (Lapchak and De Taboada, 2010). Indeed PW R/NIR-IT leads to increases in cortical ATP that are significantly higher than those seen in naïve animals. These results are compatible with the hypothesis that mitochondrial cytochrome *c* oxidase is a potent chromophore for 808-nm irradiation energy (Streeter et al., 2004), as described in detail for other disease states above.

Pre-clinical rodent studies

Given the therapeutic benefits of R/NIR-IT in a myocardial infarction setting and its promising results after application in stroke models in rabbits, Oron and his colleagues studied the effects of transcranial R/NIR-IT initiated 4 and 24 h after a middle cerebral artery occlusion (MCAO) stroke in rats (Oron et al., 2006). Using both CW and PW modes of R/NIR-IT at 7.5 mW/cm^2, they found that R/NIR-IT significantly attenuates neurological deficits in CW laser-treated rats, without decreasing stroke lesion volume when administered at 24 h, but not 4 h after the onset of a stroke (Oron et al., 2006). Unlike the neuroprotective effects of R/NIR-IT demonstrated in the RSCEM, the authors found no early neuroprotective benefit of R/NIR-IT when administered at 4 h after a stroke. Furthermore, both PW and CW R/NIR-IT have similar effects on motor outcome, although only the CW group reach significance in comparison to non-treated animals. This contrasted with an earlier study (Lapchak, 2010), the disparity perhaps attributable to the use of different experimental models and species. Interestingly, Oron et al. (2006) proposed another potential mechanism of action of R/NIR-IT, with immunocytochemical analysis of the brain post-treatment revealing increased markers of neurogenesis, which may increase the functionality of neuronal circuits and promote neuronal survival within this penumbral region (Lapchak, 2010). Others have agreed in principle with this finding, noting the 2–4 week delay in neurological outcome improvement evident post-stroke in rat models (Detaboada et al., 2006, Shen et al., 2008). Further investigations (Detaboada et al., 2006) revealed that the location of laser treatment does not affect its efficacy, with ipsilateral, contralateral or bilateral laser treatment (CW 808 nm, 7.5 mW/cm^2) administered 24 h post-stroke efficiently improving neurological outcome. Furthermore, marked and significant improvements in neurological deficits are evident at 14, 21 and 28 days post-stroke. Additional proposed mechanisms of action of R/NIR-IT in preventing neuronal death include irradiation-mediated upregulation of anti-apoptotic proteins (Liang et al., 2008), heat shock proteins and antioxidant enzymes, upregulation of the neuroprotective agent transforming growth factor beta 1 (TGF-β1) and suppression of the potentially neurotoxic agent nitric oxide synthase (NOS) (Leung et al., 2002). In summary, the beneficial effects of R/NIR-IT seen following stroke appear far reaching and are likely achieved by attenuation of several processes in concert.

It is important to note that safety studies in rodents investigating the possible short and long-term adverse neurological effects of R/NIR-IT given at different power densities and frequencies have been conducted. A diode laser (808-nm wavelength) used to deliver power densities of 7.5, 75 or 750 mW/cm^2 in either CW or PW modes results in no discernible damage to tissue and no difference between laser-treated and control groups up to 70 days post-treatment. The only rats showing adverse neurological effects are those in the CW 750 mW/cm^2 group (an approx. 100-fold increase over the current/optimal dose) (Ilic et al., 2006).

Acute ischaemic stroke clinical trials

Two randomised double-blind clinical trials with R/NIR-IT, NeuroThera® Effectiveness and Safety Trials 1 and 2 (NEST-1 and NEST-2) have already been completed (Lampl et al., 2007; Zivin et al., 2009) and because pooled results indicate a clinical improvement, a third trial (NEST-3) is currently underway. The phase II NEST-1 was a prospective, randomised 2:1, double-blinded, placebo-controlled, international and multicenter trial involving 120 ischaemic stroke patients [79 in the active treatment group and 41 shams (Lampl et al., 2007)]. Its primary aim was to assess the safety and efficacy of R/NIR-IT administered within 24 h of onset of stroke symptoms. The low-energy lasers with a wavelength of 808 nm were applied at 20 locations on the scalp with 2 min of irradiation at each site, for a power density of 10 mW/cm^2 and energy density of 1.2 J/cm^2. Mean time to treatment was over 16 h (ranging from 2 to 24 h). The primary endpoint was the National Institutes of Health Stroke Scale (NIHSS) score collapsed

into a binary outcome and the modified Rankin scale (mRS). Briefly, patients receiving R/NIR-IT had a higher proportion of positive NIHSS and mRS outcomes than did sham-treated patients (Lampl et al., 2007), and therefore this trial provides some indication that R/NIR-IT is useful in treating the motor function deficits resulting from ischaemic strokes. Furthermore, no adverse outcomes could be attributed to the laser therapeutic procedure.

The larger phase III NEST-2 trial was conducted in 660 stroke victims (Zivin et al., 2009). This was an acute ischaemic stroke study within 24 h of stroke onset and excluded patients who had received thrombolytic therapy and patients with evidence of intracerebral haemorrhage. The trial results did not reach statistical significance (p=0.094) and were considered not to be positive when all patients were included. However, post-hoc subgroup analysis detected significant improvements at 90 days (p<0.04) in the moderately impaired stroke patients (n=434) that were not evident in the severely impaired patients. Pooled analysis of the 778 patients from the NEST-1 and NEST-2 trials revealed a significant improvement in those patients treated with laser therapy (Stemer et al., 2010).

The NEST-3 clinical trial design is very similar to the NEST-2 trial and proposes to include 1000 patients within 24 h of a stroke with an NIHSS baseline of 7–17, which is the range where beneficial effects of R/NIR-IT were seen in the NEST-1 and NEST-2 trials (Lampl et al., 2007; Zivin et al., 2009). Patients have begun to be enrolled in the NEST-3 trial with the aim of demonstrating the safety and efficacy of TLT with the NeuroThera® Laser System in the treatment of subjects diagnosed with acute ischemic stroke. The initiation of the R/NIR-IT procedure must be feasible for each subject between 4.5 and 24 h of stroke onset. The earliest treatment time of 4.5 h is in agreement with the recently expanded 4.5 h therapeutic window data for tPA (Lansberg et al., 2009). Patients with infarcts located exclusively in the brainstem, cerebellum or who have small deep infarctions or massive hemispheric strokes are excluded from this trial, inferring that trial enrolment may be limited to stroke patients with only small superficial cortical infarcts, perhaps owing to the limitations of the CW R/NIR-IT regimen (Lapchak, 2012).

Interestingly, both the NEST-1 (Lampl et al., 2007) and NEST-2 (Zivin et al., 2009) trials used a CW treatment regimen with a power density similar to preclinical rabbit studies (Lapchak and De Taboada, 2010). This CW method is also currently in use in the NEST-3 trial. Perhaps given the fact that the CW R/NIR-IT regimen was not effective in all patients in the NEST-2 trial and that some preclinical studies demonstrated greater efficacy of the PW R/NIR-IT method, the CW method employed may not be optimal (Lapchak, 2012). A thorough review of the NEST trials leads to the conclusion that, based upon the translational stroke results and experimental studies in other neurodegenerative conditions (De Taboada et al., 2011), laser devices in the future should incorporate PW modes to provide optimal photobiostimulation (Lapchak, 2012). Based upon the scientific justification presented in this review, there is little doubt that R/NIR-IT should be pursued as a potential non-invasive neuroprotective treatment for ischaemic stroke patients. However, the highly novel NEST trials have not taken into account many important factors such as PW utilisation, dosimetry and adequate tissue coverage (Lapchak, 2012). Results from the NEST-3 trial are much anticipated and may pave the way for future studies, potentially incorporating PW R/NIR-IT.

R/NIR-IT for treatment of PD

PD is currently the most common movement disorder worldwide, affecting 1% to 2% of the population over the age of 60 and approximately 5% of people over the age of 85 (Alves et al., 2008). Interventions that can reduce this escalating disease burden are therefore urgently required. Although regarded as a multifactorial disorder triggered by a combination of age, genetic, environmental and other factors, mitochondrial dysfunction has consistently been implicated in the disease and is widely considered as a potential unifying factor (Banerjee et al., 2009). Consistent with this, a number of experimental models of the disease are based on inhibition of the mitochondrial respiratory chain function [using rotenone or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)], resulting in the depletion of intracellular ATP levels and the generation of free radicals. Accordingly, interventions targeted at improving mitochondrial function, such as R/NIR-IT, are highly attractive as potential treatments in PD.

In a series of *in vitro* experiments utilising striatal and cortical neurones exposed to a variety of mitochondrial toxins (cyanide, rotenone and MPTP), Wong-Riley and colleagues (Liang et al., 2006, 2008; Ying et al., 2008) demonstrated that treatment with R/NIR irradiation (670-nm LED for 80 sec, 50 mW/cm^2; 4 J/cm^2) increases cytochrome *c* oxidase activity and reduces the production of ROS/RNS. This is associated with a preservation of ATP content as well as a reduction in toxin-induced apoptosis. They also note in normal control tissue that the irradiation therapy increases cytochrome *c* oxidase activity and ATP content (Liang et al., 2008), consistent with the view

that energy absorption by the mitochondrial chromophores will increase ATP production (Eells et al., 2003) by delivering protons across the mitochondrial membrane and generating a transmembrane proton-motive force (Lapchak, 2012). Increasing ATP content will presumably facilitate the ability of the neurons to combat the effect of the neurotoxins. Notably in the neurotoxin experiments, pretreatment is more effective than treatment during toxin exposure (Ying et al., 2008), presumably reflecting the advantage of having increased ATP stores prior to toxin exposure.

A subsequent series of experiments utilising *in vivo* models of PD also demonstrated beneficial effects of R/NIR-IT on histological outcome measures. In a rapid-onset model of MPTP challenge in mice, R/NIR-IT (670-nm LED for 90 s, 50 mW/cm^2; 5 J/cm^2) administered immediately after the neurotoxin increases survival of dopaminergic neurons in the *substantia nigra pars compacta* by 35%–45% (Shaw et al., 2010). Similar results are observed in a chronic MPTP model designed to replicate the slow progressive nature of human PD. Specifically, R/NIR-IT increases the survival of dopaminergic cells in the substantia nigra pars compacta by up to 25%, despite being administered during the 3-week survival period after the 5-week MPTP insult period (Peoples et al., 2012b). No effects are observed on other dopaminergic neurons in the periaqueductal grey matter or *zona incerta*-hypothalamus, which suggests that the beneficial effects of R/NIR irradiation exposure are likely to improve motor function in PD, rather than other non-motor abnormalities such as sleep-wake cycles. Nonetheless, there are neuroprotective effects of R/NIR-IT on dopaminergic cells in the retina in both an acute and chronic MPTP-based murine model of PD (Peoples et al., 2012a). In these experiments, R/NIR-IT via LED protects cells from degeneration when administered simultaneously with the toxin, as well as rescues cells when exposure is administered after the toxin (during the 3-week survival period after 5 weeks of MPTP injections). The authors propose that the irradiation treatment stimulates the release of melatonin, a well-known and powerful antioxidant that is localised to dopaminergic neurons.

Other reports suggest that the antioxidant action may also involve the normalisation of levels of a number of antioxidant enzymes, including superoxide dismutase and catalase (Komel'kova et al., 2004). In their studies of PD patients, these authors also demonstrated that R/NIR-IT normalized blood levels of monoamine oxidase B, which catalyzes dopamine oxidation, in addition to superoxide dismutase and catalase. Whether the normalisation of blood levels reflects the normalisation of brain levels of these enzymes is unclear and requires further investigation.

Although the effects of R/NIR-IT on antioxidant enzymes have been widely reported, other mechanisms of action may also play a role in improving outcomes in experimental PD. R/NIR irradiation has been shown to promote the production of neurotrophic factors (Leung et al., 2002), as well as to suppress inflammation, specifically the production of IL-1B, TNFα and TGF (De Taboada et al., 2011). Whether these reported effects account for the positive effects of R/NIR-IT on neurogenesis is currently unknown (Oron et al., 2006); although both neurotrophic factors and inflammation are widely recognised as having modulatory effects on cell survival. These and the previously discussed mechanisms of action suggest that R/NIR-IT may thus represent a pleiotropic intervention that has been widely called for in the treatment of both acute and chronic conditions of the CNS.

Aside from the early studies of blood enzyme levels in PD (Komel'kova et al., 2004), few studies have investigated the effects of R/NIR-IT in a clinical setting for this disease. Nonetheless, several patents have been lodged over the past decade in an effort to facilitate such translation. A series of patents by Streeter and De Taboada (2012) describe the use of R/NIR-IT on PD patients through the scalp and skull to the brain more generally, whereas Di Mauro and colleagues (Di Mauro et al., 2007; Toselli et al., 2009) describe using an implantable probe to deliver R/NIR-IT locally to the substantia nigra. It has yet to be determined which approach is the most efficacious.

Clinical application of R/NIR-IT

It was originally thought that lasers (coherent, monochromatic) were essential to achieving therapeutic efficacy (Mester et al., 1985). However, the advent of LEDs (non-coherent and with a wider bandwidth), a semiconductor irradiation source that releases energy in the form of photons, provided cheaper alternatives (Posten et al., 2005) and enabled rapid uptake in a large number of human studies and randomised controlled trials (RCTs). Nevertheless, lasers currently remain the predominant irradiation source for R/NIR-IT of stroke, SCI and TBI. At last count, 112 RCTs and clinical studies have been published since 1994 on an extraordinary variety of conditions, including osteoarthritis and rheumatoid arthritis (Christie et al., 2007; Hegedus et al., 2009), carpal tunnel syndrome (Tascioglu et al., 2010), oral mucositis for chemotherapy patients (Cauwels and Martens, 2011;

Silva et al., 2011), neck pain (Chow et al., 2009) and leg ulcers (Kaviani et al., 2011). By contrast, only a small number of RCTs have been published for neurological conditions (Table 2). Efficacy has been reported for stroke (Lampl et al., 2007; Zivin et al., 2009; Stemer et al., 2010), as described above, along with three case reports for TBI (Naeser et al., 2011; Nawashiro et al., 2012) and a study on major depression (Schiffer et al., 2009). However, across the broad range of conditions that have been examined, clinical efficacy is not always clear cut, with many reports showing no benefits, for example, oral mucositis (Gouvêa de Lima et al., 2012), leg ulcers (Kokol et al., 2005), stroke (Zivin et al., 2009), pain and joint disorders (Bjordal et al., 2003) and tinnitus (Teggi et al., 2009). Information from the far larger number of RCTs and clinical studies for non-neurological conditions may offer insights into published studies, current RCTs on neurological conditions and aid in the optimal design of low irradiation laser therapy treatments for a broader range of neurological dysfunctions.

Regardless of the irradiation source (laser or LED), the dosimetry of R/NIR-IT is highly complex because a wide range of parameters can be altered, including wavelength, irradiance, pulse structure, coherence and polarisation, as well as the actual dose delivered, which can involve variations in energy, energy density, irradiation time and treatment interval in addition to the site of the injury or disease (Chung et al., 2012). Highly variable dosimetry between studies makes direct comparison difficult if not impossible (Jenkins and Carroll, 2011) and has contributed to lack of consensus as well as scepticism regarding efficacy.

Indeed, for conditions that have been widely studied, three *Cochrane Database Systematic Reviews* reveal conflicting data for osteoarthritis [5 RCTs, 112 patients (Brosseau et al., 2003b)], some benefit for rheumatoid arthritis [5 RCTs, 222 patients (Brosseau et al., 2003a)] and insufficient data to draw conclusions for low-back pain [6 RCTs, 318 patients (Yousefi-Nooraie et al., 2008)]. The Cochrane Reviews points to a lack of standardised, validated outcomes, lack of harmonised dose calculation and an absence of data on how effectiveness is affected by wavelength, treatment duration, dosage and site of application. The recent call to harmonise reporting of R/NIR-IT suggests mandatory inclusion of eight beam parameters (wavelength, power, irradiation time, beam area, pulse parameters, anatomical location, number of treatments and the interval between treatments) as well as other details for the reporting of both clinical and laboratory studies (Jenkins and Carroll, 2011).

An interrelated dosimetry issue is that of the biphasic dose response in biological tissue that is observed both *in vitro* and *in vivo*, which is characterised by initial efficacy as irradiance and time are increased, followed by a decline, no effect or even inhibition (Huang et al., 2011; Chung et al., 2012). The mechanism underpinning the biphasic response is thought to involve ROS/RNS that are normally produced at low levels in healthy cells and are key signalling transcription factors (Shi and Gibson, 2007; Leonarduzzi et al., 2011). Low R/NIR-IT doses result in cellular events such as proliferation, migration and neurite outgrowth *in vitro* as well as improvements in various conditions *in vivo*, such as wound healing, cardiac infarction and arthritis. However, higher doses, which result in excessive ROS, show reduced effects or inhibition (reviewed by Hashmi et al., 2010; Huang et al., 2011).

In this context and as described earlier, Huang et al. (2011) have shown in a mouse pneumatic cortical impact model that delivering a single dose (36 J/cm^2 810-nm laser at 50 mW/cm^2) over 12 min is beneficial on the NSS but that a 10-fold greater dose (360 J/cm^2 810-nm laser at 500 mW/cm^2) also delivered over 12 min results in worse outcomes compared to no treatment. Intriguingly, delivering the same

Table 2 Currently registered trials on the WHO International Clinical Trials Registry Platform Search Portal.

Title	Registration date	Status
Brain plasticity underlying back pain response to different acupuncture methods	5/2012	NR
Effects of LEDs on memory in TBI patients	5/2012	R
Transcranial laser therapy in the rehabilitation of hemiplegic patients from ischaemic stroke	3/2011	NR
Safety of Rt-PA + transcranial emission of low energy lasers for acute stroke recovery	10/2010	R
Efficacy and safety trial of transcranial laser therapy within 24 h from stroke onset (NEST-3)	5/2010	R
Brain effects of acupuncture 2: laser acupuncture vs. laser EMLA	4/2010	NR
Brain effects of acupuncture 2: needle acupuncture vs. laser acupuncture	4/2010	NR
Managing fatigue and sleep disturbance following traumatic brain injury	7/2008	R
Managing fatigue and sleep disturbance following traumatic brain injury	1/2008	NR
Effectiveness and safety trial of a new ischaemic stroke treatment within 24 h from stroke onset (NEST-2)	1/2007	NR

R, recruiting; NR, not recruiting.

low dose daily for 14 days starting at 4 h after injury results in a slight improvement at day 4 compared to the single dose but, between days 14–28, NSS is no better than with no treatment and shows a trend to worse outcomes. The biphasic tissue response cautions that 'more is not necessarily better'. As we argue above, this conclusion is supported by the fact that only extremely low irradiation doses are sufficient to elicit biophysical changes at the cellular level. Nevertheless, the almost complete lack of adverse/serious adverse events reported in clinical trials argues for continued clinical investigation of R/NIR-IT.

In addition to relative safety, the non-invasive nature of R/NIR-IT has undoubtedly contributed to its relatively rapid uptake in clinical trials. For example, R/NIR laser therapy was developed and patented between 1997 and 2001, with the first clinical trial in acute ischaemic stroke published in 2007 (NEST-1) (Lampl et al., 2007). This was followed by NEST-2 (Zivin et al., 2009), an analysis of pooled data from NEST-1 and NEST-2 (Stemer et al., 2010) and NEST-3 now underway (Lapchak, 2010). With respect to the future, there are 10 currently registered RCTs for R/NIR-IT in neurological conditions, one of which is published (NEST-2). The hope is that these will yield data that avoid the shortcomings highlighted by the Cochrane Reviews on R/NIR-IT in other conditions (Brosseau et al., 2003a,b; Yousefi-Nooraie et al., 2008) and progress the field regarding the use of R/NIR-IT.

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References

Ad, N. and Oron, U. (2001). Impact of low level laser irradiation on infarct size in the rat following myocardial infarction. Int. J. Cardiol. *80*, 109–116.

Age-Related Eye Disease Study Group. (2001). A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch. Ophthalmol. *119*, 1439–1452.

Albarracin, R. and Valter, K. (2012a). 670 nm red light preconditioning supports Müller cell function: evidence from the white light-induced damage model in the rat retina. Photochem. Photobiol. *88*, 1418–1427.

Albarracin, R.S. and Valter, K. (2012b). Treatment with 670-nm light protects the cone photoreceptors from white light-induced degeneration. Adv. Exp. Med. Biol. *723*, 121–128.

Albarracin, R., Eells, J., and Valter, K. (2011). Photobiomodulation protects the retina from light-induced photoreceptor degeneration. Invest. Ophthalmol. Vis. Sci. *52*, 3582–3592.

Alves, G., Forsaa, E.B., Pedersen, K.F., Dreetz Gjerstad, M., and Larsen, J.P. (2008). Epidemiology of Parkinson's disease. J. Neurol. *255*(Suppl. 5), 18–32.

Ando, T., Xuan, W., Xu, T., Dai, T., Sharma, S.K., Kharkwal, G.B., Huang, Y.Y., Wu, Q., Whalen, M.J., Sato, S., et al. (2011). Comparison of therapeutic effects between pulsed and continuous wave 810-nm wavelength laser irradiation for traumatic brain injury in mice. PLoS One *6*, e26212.

Ankri, R., Lubart, R., and Taitelbaum, H. (2010). Estimation of the optimal wavelengths for laser-induced wound healing. Lasers Surg. Med. *42*, 760–764.

Assia, E., Rosner, M., Belkin, M., Solomon, A., and Schwartz, M. (1989). Temporal parameters of low energy laser irradiation for optimal delay of post-traumatic degeneration of rat optic nerve. Brain Res. *476*, 205–212.

Balaratnasingam, C., Morgan, W.H., Johnstone, V., Cringle, S.J., and Yu, D.Y. (2009). Heterogeneous distribution of axonal cytoskeleton proteins in the human optic nerve. Invest. Ophthalmol. Vis. Sci. *50*, 2824–2838.

Ball, K.A., Castello, P.R., and Poyton, R.O. (2011). Low intensity light stimulates nitrite-dependent nitric oxide synthesis but not oxygen consumption by cytochrome c oxidase: implications for phototherapy. J. Photochem. Photobiol. B *102*, 182–191.

Banerjee, R., Starkov, A.A., Beal, M.F., and Thomas, B. (2009). Mitochondrial dysfunction in the limelight of Parkinson's disease pathogenesis. Biochim. Biophys. Acta *1792*, 651–663.

Baptiste, D.C. and Fehlings, M.G. (2006). Pharmacological approaches to repair the injured spinal cord. J. Neurotrauma *23*, 318–334.

Biron, K.E., Dickstein, D.L., Gopaul, R., and Jefferies, W.A. (2011). Amyloid triggers extensive cerebral angiogenesis causing blood brain barrier permeability and hypervascularity in Alzheimer's disease. PloS One *6*, e23789.

Bjordal, J.M., Couppé, C., Chow, R.T., Tunér, J., and Ljunggren, E.A. (2003). A systematic review of low level laser therapy with location-specific doses for pain from chronic joint disorders. Aust. J. Physiother. *49*, 107–116.

Brambilla, R., Bracchi-Ricard, V., and Bethea, J.R. (2006). Cytokines in brain and spinal cord injury. In: Cytokines and the CNS. R.M. Ransohoff and E.N. Benveniste, eds. (Boca Raton, FL: CRC Publications), pp. 299–326.

Brosseau, L., Judd, M.G., Marchand, S., Robinson, V.A., Tugwell, P., Wells, G., and Yonge, K. (2003a). Transcutaneous electrical nerve stimulation (TENS) for the treatment of rheumatoid arthritis in the hand. Cochrane Database Syst. Rev. 2003, CD004377.

Brosseau, L., Welch, V., Wells, G., deBie, R., Gam, A., Harman, K., Morin, M., Shea, B., and Tugwell, P. (2003b). Low level

laser therapy (Classes I, II and III) for treating osteoarthritis. Cochrane Database Syst. Rev. 2003, CD002046.

Buki, A., Okonkwo, D.O., and Povlishock, J.T. (1999). Postinjury cyclosporin A administration limits axonal damage and disconnection in traumatic brain injury. J. Neurotrauma *16*, 511–521.

Byrnes, K.R., Waynant, R.W., Ilev, I.K., Wu, X., Barna, L., Smith, K., Heckert, R., Gerst, H., and Anders, J.J. (2005). Light promotes regeneration and functional recovery and alters the immune response after spinal cord injury. Lasers Surg. Med. *36*, 171–185.

Camello-Almaraz, M.C., Pozo, M.J., Murphy, M.P., and Camello, P.J. (2006). Mitochondrial production of oxidants is necessary for physiological calcium oscillations. J. Cell Physiol. *206*, 487–494.

Carter, K. and Palmer, G. (1982). Models of the two heme centers in cytochrome oxidase. The optical properties of cytochrome a and a3. J. Biol. Chem. *257*, 13507–13514.

Cauwels, R.G. and Martens, L.C. (2011). Low level laser therapy in oral mucositis: a pilot study. Eur. Arch. Paediatr. Dent. *12*, 118–123.

Cheong, W.F., Prahl, S.A., and Welch, A.J. (1990). A review of the optical properties of biological tissues. IEEE J. Quantum Electronics *26*, 2166–2185.

Chow, R.T., Johnson, M.I., Lopes-Martins, R.A., and Bjordal, J.M. (2009). Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomised placebo or active-treatment controlled trials. Lancet *374*, 1897–1908.

Christie, A., Jamtvedt, G., Dahm, K.T., Moe, R.H., Haavardsholm, E.A., and Hagen, K.B. (2007). Effectiveness of nonpharmacological and nonsurgical interventions for patients with rheumatoid arthritis: an overview of systematic reviews. Phys. Ther. *87*, 1697–1715.

Chung, H., Dai, T., Sharma, S.K., Huang, Y.Y., Carroll, J.D., and Hamblin, M.R. (2012). The nuts and bolts of low-level laser (light) therapy. Ann. Biomed. Eng. *40*, 516–533.

Curcio, C.A., Sloan, K.R., Kalina, R.E., and Hendrickson, A.E. (1990). Human photoreceptor topography. J. Comp. Neurol. *292*, 497–523.

De Taboada, L., Yu, J., El-Amouri, S., Gattoni-Celli, S., Richieri, S., McCarthy, T., Streeter, J., and Kindy, M.S. (2011). Transcranial laser therapy attenuates amyloid-beta peptide neuropathology in amyloid-beta protein precursor transgenic mice. J. Alzheimers Dis. *23*, 521–535.

Demontis, G.C., Longoni, B., and Marchiafava, P.L. (2002). Molecular steps involved in light-induced oxidative damage to retinal rods. Invest. Ophthalmol. Vis. Sci. *43*, 2421–2427.

Desai Bradaric, B., Patel, A., Schneider, J.A., Carvey, P.M., and Hendey, B. (2012). Evidence for angiogenesis in Parkinson's disease, incidental Lewy body disease, and progressive supranuclear palsy. J. Neural Transm. *119*, 59–71.

Detaboada, L., Ilic, S., Leichliter-Martha, S., Oron, U., Oron, A., and Streeter, J. (2006). Transcranial application of low-energy laser irradiation improves neurological deficits in rats following acute stroke. Lasers Surg. Med. *38*, 70–73.

Di Mauro, T.M., Attawia, M., Holy, C., Lilienfeld, S., Sutton, J.K., and Ward, M. (2007). Red light implant for treating Parkinson's disease. U.S. Patent 7288108. Filing date March 14, 2005. Issue date October 30, 2007.

Eells, J.T., Henry, M.M., Summerfelt, P., Wong-Riley, M.T., Buchmann, E.V., Kane, M., Whelan, N.T., and Whelan, H.T. (2003). Therapeutic photobiomodulation for methanol-induced retinal toxicity. Proc. Natl. Acad. Sci. USA *100*, 3439–3444.

Eells, J.T., Wong-Riley, M.T., VerHoeve, J., Henry, M., Buchman, E.V., Kane, M.P., Gould, L.J., Das, R., Jett, M., Hodgson, B.D., et al. (2004). Mitochondrial signal transduction in accelerated wound and retinal healing by near-infrared light therapy. Mitochondrion *4*, 559–567.

Eichler, J., Knof, J., and Lenz, H. (1977). Measurements on depth of penetration of light (0.35–1.0mu-M) in tissue. Radiat. Env. Biophys. *14*, 239–242.

Firbank, M., Hiraoka, M., Essenpreis, M., and Delpy, D.T. (1993). Measurement of the optical properties of the skull in the wavelength range 650–950 nm. Phys. Med. Biol. *38*, 503–510.

Fitzgerald, M., Bartlett, C.A., Payne, S.C., Hart, N.S., Rodger, J., Harvey, A.R., and Dunlop, S.A. (2010). Near infrared light reduces oxidative stress and preserves function in CNS tissue vulnerable to secondary degeneration following partial transection of the optic nerve. J. Neurotrauma *27*, 2107–2119.

Gouvêa de Lima, A., Villar, R.C., de Castro, G. Jr, Antequera, R., Gil, E., Rosalmeida, M.C., Federico, M.H., and Snitcovsky, I.M. (2012). Oral mucositis prevention by low-level laser therapy in head-and-neck cancer patients undergoing concurrent chemoradiotherapy: a phase III randomized study. Int. J. Rad. Oncol. Biol. Phys. *82*, 270–275.

Hartz, A.M., Bauer, B., Soldner, E.L., Wolf, A., Boy, S., Backhaus, R., Mihaljevic, I., Bogdahn, U., Klünemann, H.H., Schuierer, G., et al. (2012). Amyloid-beta contributes to blood-brain barrier leakage in transgenic human amyloid precursor protein mice and in humans with cerebral amyloid angiopathy. Stroke *43*, 514–523.

Hashmi, J.T., Huang, Y.Y., Osmani, B.Z., Sharma, S.K., Naeser, M.A., and Hamblin, M.R. (2010). Role of low-level laser therapy in neurorehabilitation. PM. R. *2*(12 Suppl. 2), 292–305.

Hebeda, K.M., Menovsky, T., Beek, J.F., Wolbers, J.G., and van Gemert, M.J. (1994). Light propagation in the brain depends on nerve fiber orientation. Neurosurgery *35*, 720–722; discussion 722–724.

Hegedus, B., Viharos, L., Gervain, M., and Gálfi, M. (2009). The effect of low-level laser in knee osteoarthritis: a double-blind, randomized, placebo-controlled trial. Photomed. Laser Surg. *27*, 577–584.

Hollyfield, J.G., Bonilha, V.L., Rayborn, M.E., Yang, X., Shadrach, K.G., Lu, L., Ufret, R.L., Salomon, R.G., and Perez, V.L. (2008). Oxidative damage-induced inflammation initiates age-related macular degeneration. Nat. Med. *14*, 194–198.

Hom, J., Dankbaar, J.W., Soares, B.P., Schneider, T., Cheng, S.C., Bredno, J., Lau, B.C., Smith, W., Dillon, W.P., and Wintermark, M. (2011). Blood-brain barrier permeability assessed by perfusion CT predicts symptomatic hemorrhagic transformation and malignant edema in acute ischemic stroke. Am. J. Neuroradiol. *32*, 41–48.

Howards, R., Holmes, P.A., Siddiqui, A., Treacher, D., Tsiropoulos, I., and Koutroumanidis, M. (2012). Hypoxic-ischemic brain injury: imaging and neurophysiological abnormalities related to outcome. Q. J. Med. *105*, 551–561.

Huang, Y.Y., Sharma, S.K., Carroll, J., and Hamblin, M.R. (2011). Biphasic dose response in low level light therapy – an update. Dose Response *9*, 602–618.

Huang, Y.Y., Gupta, A., Vecchio, D., de Arce, V.J., Huang, S.F., Xuan, W., and Hamblin, M.R. (2012). Transcranial low level laser (light) therapy for traumatic brain injury. J. Biophotonics *5*, 827–837.

Ilic, S., Leichliter, S., Streeter, J., Oron, A., DeTaboada, L., and Oron, U. (2006). Effects of power densities, continuous and pulse frequencies, and number of sessions of low-level laser therapy on intact rat brain. Photomed. Laser Surg. *24*, 458–466.

Ishiguro, M., Ikeda, K., and Tomita, K. (2010). Effect of near-infrared light-emitting diodes on nerve regeneration. J. Orthop. Sci. *15*, 233–239.

Iwamura, A., Taoka, T., Fukusumi, A., Sakamoto, M., Miyasaka, T., Ochi, T., Akashi, T., Okuchi, K., and Kichikawa, K. (2012). Diffuse vascular injury: convergent-type hemorrhage in the supratentorial white matter on susceptibility-weighted image in cases of severe traumatic brain damage. Neuroradiology *54*, 335–343.

Jenkins, P.A. and Carroll, J.D. (2011). How to report low-level laser therapy (LLLT)/photomedicine dose and beam parameters in clinical and laboratory studies. Photomed. Laser Surg. *29*, 785–787.

Johnson, V.E., Stewart, W., and Smith, D.H. (2012). Axonal pathology in traumatic brain injury. Exp. Neurol. January 20, in press.

Karu, T. (1989). Photobiology of low-power laser effects. Health Phys. *56*, 691–704.

Karu, T. (1999). Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J. Photochem. Photobiol. B *49*, 1–17.

Karu, T.I. and Kolyakov, S.F. (2005). Exact action spectra for cellular responses relevant to phototherapy. Photomed. Laser Surg. *23*, 355–361.

Karu, T.I., Pyatibrat, L.V., Kolyakov, S.F., and Afanasyeva, N.I. (2005). Absorption measurements of a cell monolayer relevant to phototherapy: reduction of cytochrome c oxidase under near IR radiation. J. Photochem. Photobiol. B *81*, 98–106.

Karu, T.I., Pyatibrat, L.V., Kolyakov, S.F., and Afanasyeva, N.I. (2008). Absorption measurements of cell monolayers relevant to mechanisms of laser phototherapy: reduction or oxidation of cytochrome c oxidase under laser radiation at 632.8 nm. Photomed. Laser Surg. *26*, 593–599.

Kaviani, A., Djavid, G.E., Ataie-Fashtami, L., Fateh, M., Ghodsi, M., Salami, M., Zand, N., Kashef, N., and Larijani, B. (2011). A randomized clinical trial on the effect of low-level laser therapy on chronic diabetic foot wound healing: a preliminary report. Photomed. Laser Surg. *29*, 109–114.

Keane, R.W., Davis, A.R., and Dietrich, W.D. (2006). Inflammatory and apoptotic signaling after spinal cord injury. J. Neurotrauma *23*, 335–344.

Khuman, J., Zhang, J., Park, J., Carroll, J.D., Donahue, C., and Whalen, M.J. (2012). Low-level laser light therapy improves cognitive deficits and inhibits microglial activation after controlled cortical impact in mice. J. Neurotrauma *29*, 408–417.

Kokkinopoulos, I., Colman, A., Hogg, C., Heckenlively, J., and Jeffery, G. (2012). Age-related retinal inflammation is reduced by 670 nm light via increased mitochondrial membrane potential. Neurobiol. Aging *34,* 602–609.

Kokol, R., Berger, C., Haas, J., and Kopera, D. (2005). [Venous leg ulcers: no improvement of wound healing with 685-nm low level laser therapy. Randomised, placebo-controlled, double-blind study]. Hautarzt *56*, 570–575.

Komel'kova, L.V., Vitreshchak, T.V., Zhirnova, I.G., Poleshchuk, V.V., Stvolinskiĭ, S.L., Mikhaĭlov, V.V., Gannushkina, I.V., and Piradov, M.A. (2004). [Biochemical and immunological induces of the blood in Parkinson's disease and their correction with the help of laser therapy]. Patol. Fiziol. Eksp. Ter. *1*, 15–18.

Kowaltowski, A.J., de Souza-Pinto, N.C., Castilho, R.F., and Vercesi, A.E. (2009). Mitochondria and reactive oxygen species. Free. Radic. Biol. Med. *47*, 333–343.

Lampl, Y., Zivin, J.A., Fisher, M., Lew, R., Welin, L., Dahlof, B., Borenstein, P., Andersson, B., Perez, J., and Caparo, C. (2007). Infrared laser therapy for ischemic stroke: a new treatment strategy: results of the NeuroThera Effectiveness and Safety Trial-1 (NEST-1). Stroke *38*, 1843–1849.

Lansberg, M.G., Bluhmki, E., and Thijs, V.N. (2009). Efficacy and safety of tissue plasminogen activator 3 to 4.5 hours after acute ischemic stroke: a metaanalysis. Stroke *40*, 2438–2441.

Lapchak, P.A. (2010). Taking a light approach to treating acute ischemic stroke patients: transcranial near-infrared laser therapy translational science. Ann. Med. *42*, 576–586.

Lapchak, P.A. (2012). Transcranial near-infrared laser therapy applied to promote clinical recovery in acute and chronic neurodegenerative diseases. Expert Rev. Med. Devices *9*, 71–83.

Lapchak, P.A. and De Taboada, L. (2010). Transcranial near infrared laser treatment (NILT) increases cortical adenosine-5'-triphosphate (ATP) content following embolic strokes in rabbits. Brain Res *1306*, 100–105.

Lapchak, P.A., Araujo, D.M., Song, D., Wei, J., and Zivin, J.A. (2002). Neuroprotective effects of the spin trap agent disodium-[(tert-butylimino)methyl]benzene-1,3-disulfonate N-oxide (generic NXY-059) in a rabbit small clot embolic stroke model: combination studies with the thrombolytic tissue plasminogen activator. Stroke *33*, 1411–1415.

Lapchak, P.A., Salgado, K.F., Chao, C.H., and Zivin, J.A. (2007). 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 *148*, 907–914.

Lapchak, P.A., Han, M.K., Salgado, K.F., Streeter, J., and Zivin, J.A. (2008). Safety profile of transcranial near-infrared laser therapy administered in combination with thrombolytic therapy to embolized rabbits. Stroke *39*, 3073–3078.

Lenz, P. (1999). Fluorescence measurement in thick tissue layers by linear or nonlinear long-wavelength excitation. Appl. Opt. *38*, 3662–3669.

Leonarduzzi, G., Sottero, B., Testa, G., Biasi, F., and Poli. G. (2011). New insights into redox-modulated cell signaling. Curr. Pharm. Des. *17*, 3994–4006.

Leung, M.C., Lo, S.C., Siu, F.K., and So, K.F. (2002). Treatment of experimentally induced transient cerebral ischemia with low energy laser inhibits nitric oxide synthase activity and up-regulates the expression of transforming growth factor-beta 1. Lasers Surg. Med. *31*, 283–288.

Liang, H.L., Whelan, H.T., Eells, J.T., Meng, H., Buchmann, E., Lerch-Gaggl, A., and Wong-Riley, M. (2006). Photobiomodulation partially rescues visual cortical neurons from cyanide-induced apoptosis. Neuroscience *139*, 639–649.

Liang, H.L., Whelan, H.T., Eells, J.T., and Wong-Riley, M.T. (2008). Near-infrared light via light-emitting diode treatment is therapeutic against rotenone- and 1-methyl-4-phenylpyridinium ion-induced neurotoxicity. Neuroscience *153*, 963–974.

Lim, J., Ali, Z.M., Sanders, R.A., Snyder, A.C., Eells, J.T., Henshel, D.S., and Watkins, J.B. 3rd. (2009). Effects of low-level light therapy on hepatic antioxidant defense in acute and chronic diabetic rats. J. Biochem. Mol. Toxicol. *23*, 1–8.

Lim, J., Sanders, R.A., Snyder, A.C., Eells, J.T., Henshel, D.S., and Watkins, J.B. 3rd. (2010). Effects of low-level light therapy on streptozotocin-induced diabetic kidney. J. Photochem. Photobiol. B *99*, 105–110.

Liu, X.Z., Xu, X.M., Hu, R., Du, C., Zhang, S.X., McDonald, J.W., Dong, H.X., Wu, Y.J., Fan, G.S., Jacquin, M.F., et al. (1997). Neuronal and glial apoptosis after traumatic spinal cord injury. J. Neurosci. *17*, 5395–5406.

Mason, M.G., Nicholls, P., Wilson, M.T., and Cooper, C.E. (2006). Nitric oxide inhibition of respiration involves both competitive (heme) and noncompetitive (copper) binding to cytochrome c oxidase. Proc. Natl. Acad. Sci. USA *103*, 708–713.

Maxwell, W.L., Domleo, A., McColl, G., Jafari, S.S., and Graham, D.I. (2003). Post-acute alterations in the axonal cytoskeleton after traumatic axonal injury. J. Neurotrauma *20*, 151–168.

McCarthy, T.J., De Taboada, L., Hildebrandt, P.K., Ziemer, E.L., Richieri, S.P., and Streeter, J. (2010). Long-term safety of single and multiple infrared transcranial laser treatments in Sprague-Dawley rats. Photomed. Laser Surg. *28*, 663–667.

Medalha, C.C., Amorim, B.O., Ferreira, J.M., Oliveira, P., Pereira, R.M., Tim, C., Lirani-Galvão, A.P., da Silva, O.L., and Renno, A.C. (2010). Comparison of the effects of electrical field stimulation and low-level laser therapy on bone loss in spinal cord-injured rats. Photomed. Laser Surg. *28*, 669–674.

Mester, E., Mester, A.F., and Mester, A. (1985). The biomedical effects of laser application. Lasers Surg. Med. *5*, 31–39.

Mitchell, U.H., Myrer, J.W., Johnson, A.W., and Hilton, S.C. (2011). Restless legs syndrome and near-infrared light: an alternative treatment option. Physiother. Theory Pract. *27*, 345–351.

Mitra, S. and Foster, T.H. (2004). Carbogen breathing significantly enhances the penetration of red light in murine tumours in vivo. Phys. Med. Biol. *49*, 1891–1904.

Moftakhar, P., Lynch, M.D., Pomakian, J.L., and Vinters, H.V. (2010). Aquaporin expression in the brains of patients with or without cerebral amyloid angiopathy. J. Neuropathol. Exp. Neurol. *69*, 1201–1209.

Moreira, M.S., Velasco, I.T., Ferreira, L.S., Ariga, S.K., Barbeiro, D.F., Meneguzzo, D.T., Abatepaulo, F., and Marques, M.M. (2009). Effect of phototherapy with low intensity laser on local and systemic immunomodulation following focal brain damage in rat. J. Photochem. Photobiol. B *97*, 145–151.

Muller, P.J. and Wilson, B.C. (1986). An update on the penetration depth of 630 nm light in normal and malignant human-brain tissue in vivo. Phys. Med. Biol. *31*, 1295–1297.

Naeser, M.A., Saltmarche, A., Krengel, M.H., Hamblin, M.R., and Knight, J.A. (2011). Improved cognitive function after transcranial, light-emitting diode treatments in chronic, traumatic brain injury: two case reports. Photomed. Laser Surg. *29*, 351–358.

Nashmi, R. and Fehlings, M.G. (2001). Mechanisms of axonal dysfunction after spinal cord injury: with an emphasis on the role of voltage-gated potassium channels. Brain Res. Brain Res. Rev. *38*, 165–191.

Natoli, R., Zhu, Y., Valter, K., Bisti, S., Eells, J., and Stone, J. (2010). Gene and noncoding RNA regulation underlying photoreceptor protection: microarray study of dietary antioxidant saffron and photobiomodulation in rat retina. Mol. Vis. *16*, 1801–1822.

Nawashiro, H., Wada, K., Nakai, K., and Sato, S. (2012). Focal increase in cerebral blood flow after treatment with near-infrared light to the forehead in a patient in a persistent vegetative state. Photomed. Laser Surg. *30*, 231–233.

Neupane, J., Ghimire, S., Shakya, S., Chaudhary, L., and Shrivastava, V.P. (2010). Effect of light emitting diodes in the photodynamic therapy of rheumatoid arthritis. Photodiagnosis Photodyn. Ther. *7*, 44–49.

Oddo, M., Levine, J.M., Mackenzie, L., Frangos, S., Feihl, F., Kasner, S.E., Katsnelson, M., Pukenas, B., Macmurtrie, E., Maloney-Wilensky, E., et al. (2011). Brain hypoxia is associated with short-term outcome after severe traumatic brain injury independently of intracranial hypertension and low cerebral perfusion pressure. Neurosurgery *69*, 1037–1045; discussion 1045.

Oron, U., Yaakobi, T., Oron, A., Hayam, G., Gepstein, L., Rubin, O., Wolf, T., and Ben Haim, S. (2001). Attenuation of infarct size in rats and dogs after myocardial infarction by low-energy laser irradiation. Lasers Surg. Med. *28*, 204–211.

Oron, A., Oron, U., Chen, J., Eilam, A., Zhang, C., Sadeh, M., Lampl, Y., Streeter, J., DeTaboada, L., and Chopp, M. (2006). Low-level laser therapy applied transcranially to rats after induction of stroke significantly reduces long-term neurological deficits. Stroke *37*, 2620–2624.

Oron, A., Oron, U., Streeter, J., de Taboada, L., Alexandrovich, A., Trembovler, V., and Shohami, E. (2007). Low-level laser therapy applied transcranially to mice following traumatic brain injury significantly reduces long-term neurological deficits. J. Neurotrauma *24*, 651–656.

Oron, A., Oron, U., Streeter, J., De Taboada, L., Alexandrovich, A., Trembovler, V., and Shohami, E. (2012). Near infrared transcranial laser therapy applied at various modes to mice following traumatic brain injury significantly reduces long-term neurological deficits. J. Neurotrauma *20*, 401–407.

Park, E., Velumian, A.A., and Fehlings, M.G. (2004). The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J. Neurotrauma *21*, 754–774.

Parrish, J.A. (1981). New concepts in therapeutic photomedicine: photochemistry, optical targeting and the therapeutic window. J. Invest. Dermatol. *77*, 45–50.

Peng, T.I. and Jou, M.J. (2010). Oxidative stress caused by mitochondrial calcium overload. Ann. NY Acad. Sci. *1201*, 183–188.

Peoples, C., Shaw, V.E., Stone, J., Jeffery, G., Baker, G.E., and Mitrofanis, J. (2012a). Survival of dopaminergic amacrine cells after near-infrared light treatment in MPTP-treated mice. ISRN Neurol. *2012*, 850150.

Peoples, C., Spana, S., Ashkan, K., Benabid, A.L., Stone, J., Baker, G.E., and Mitrofanis, J. (2012b). Photobiomodulation enhances nigral dopaminergic cell survival in a chronic MPTP mouse model of Parkinson's disease. Parkinsonism Relat. Disord. *18*, 469–476.

Posten, W., Wrone, D.A., Dover, J.S., Arndt, K.A., Silapunt, S., and Alam, M. (2005). Low-level laser therapy for wound healing: mechanism and efficacy. Dermatol. Surg. *31*, 334–340.

Profio, A.E. (1989). Light transport in tissue. Appl. Opt. *28*, 2216–2221.

Profyris, C., Cheema, S.S., Zang, D., Azari, M.F., Boyle, K., and Petratos, S. (2004). Degenerative and regenerative mechanisms governing spinal cord injury. Neurobiol. Dis. *15*, 415–436.

Qu, C., Cao, W., Fan, Y., and Lin, Y. (2010). Near-infrared light protect the photoreceptor from light-induced damage in rats. Adv. Exp. Med. Biol. *664*, 365–374.

Quirk, B.J. and Whelan, H.T. (2011). Near-infrared irradiation photobiomodulation: the need for basic science. Photomed. Laser Surg. *29*, 143–144.

Quirk, B.J., Torbey, M., Buchmann, E., Verma, S., and Whelan, H.T. (2012). Near-infrared photobiomodulation in an animal model of traumatic brain injury: improvements at the behavioral and biochemical levels. Photomed. Laser Surg. *30*, 523–529.

Rochkind, S., Geuna, S., and Shainberg, A. (2009). Chapter 25: phototherapy in peripheral nerve injury: effects on muscle preservation and nerve regeneration. Int. Rev. Neurobiol. *87*, 445–464.

Rodieck, R.W. (1998). The First Steps in Seeing (Sunderland, MA: Sinauer Associates).

Rodríguez-Baeza, A., Reina-de la Torre, F., Poca, A., Martí, M., and Garnacho, A. (2003). Morphological features in human cortical brain microvessels after head injury: a three-dimensional and immunocytochemical study. Anat. Rec. A Discov. Mol. Cell Evol. Biol. *273*, 583–593.

Rojas, J.C., Lee, J., John, J.M., and Gonzalez-Lima, F. (2008). Neuroprotective effects of near-infrared light in an in vivo model of mitochondrial optic neuropathy. J. Neurosci. *28*, 13511–13521.

Rutar, M., Provis, J.M., and Valter, K. (2010). Brief exposure to damaging light causes focal recruitment of macrophages, and long-term destabilization of photoreceptors in the albino rat retina. Curr. Eye Res. *35*, 631–643.

Rutar, M., Natoli, R., Valter, K., and Provis, J.M. (2011). Early focal expression of the chemokine Ccl2 by Muller cells during exposure to damage-inducing bright continuous light. Invest. Ophthalmol. Vis. Sci. *52*, 2379–2388.

Sandeman, D., Mills, T., and Brown, S.G. (1986). Enhancement of light penetration by white matter tracts in the normal mouse brain. Lasers Surg. Med. *3*, 47.

Schiffer, F., Johnston, A.L., Ravichandran, C., Polcari, A., Teicher, M.H., Webb, R.H., and Hamblin, M.R. (2009). 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. *5*, 46.

Segura, T., Calleja, S., and Jordan, J. (2008). Recommendations and treatment strategies for the management of acute ischemic stroke. Expert Opin. Pharmacother. *9*, 1071–1085.

Sekhon, L.H. and Fehlings, M.G. (2001). Epidemiology, demographics, and pathophysiology of acute spinal cord injury. Spine *26*(24 Suppl.), S2–12.

Seme, M.T., Summerfelt, P., Henry, M.M., Neitz, J., and Eells, J.T. (1999). Formate-induced inhibition of photoreceptor function in methanol intoxication. J. Pharmacol. Exp. Ther. *289*, 361–370.

Shaw, V.E., Spana, S., Ashkan, K., Benabid, A.L., Stone, J., Baker, G.E., and Mitrofanis, J. (2010). Neuroprotection of midbrain dopaminergic cells in MPTP-treated mice after near-infrared light treatment. J. Comp. Neurol. *518*, 25–40.

Shen, J., Yang, X., Dong, A., Petters, R.M., Peng, Y.W., Wong, F., and Campochiaro, P.A. (2005). Oxidative damage is a potential cause of cone cell death in retinitis pigmentosa. J. Cell Physiol. *203*, 457–464.

Shen, J., Xie, L., Mao, X., Zhou, Y., Zhan, R., Greenberg, D.A., and Jin, K. (2008). Neurogenesis after primary intracerebral hemorrhage in adult human brain. J. Cereb. Blood Flow Metab. *28*, 1460–1468.

Shi, Q. and Gibson, G.E. (2007). Oxidative stress and transcriptional regulation in Alzheimer disease. Alzheimer Dis. Assoc. Disord. *21*, 276–291.

Silva, G.B., Mendonça, E.F., Bariani, C., Antunes, H.S., and Silva, M.A. (2011). The prevention of induced oral mucositis with low-level laser therapy in bone marrow transplantation patients: a randomized clinical trial. Photomed. Laser Surg. *29*, 27–31.

Stemer, A.B., Huisa, B.N., and Zivin, J.A. (2010). The evolution of transcranial laser therapy for acute ischemic stroke, including a pooled analysis of NEST-1 and NEST-2. Curr. Cardiol. Rep. *12*, 29–33.

Sterenborg, H., Van Gemert, M.J.C., Kamphorst, W., Wolbers, J.G., and Hogervorst, W. (1989). The spectral dependence of optical properties of human brain. Lasers Med. Sci. *4,* 221–227.

Stolik, S., Delgado, J.A., Pérez, A., and Anasagasti, L. (2000). Measurement of the penetration depths of red and near infrared light in human "ex vivo" tissues. J. Photochem. Photobiol. B *57*, 90–93.

Stone, J., Maslim, J., Valter-Kocsi, K., Mervin, K., Bowers, F., Chu, Y., Barnett, N., Provis, J., Lewis, G., and Fisher, S.K. (1999). Mechanisms of photoreceptor death and survival in mammalian retina. Prog. Retin. Eye Res. *18*, 689–735.

Streeter, J., De Taboada, L., and Oron, U. (2004). Mechanisms of action of light therapy for stroke and acute myocardial infarction. Mitochondrion *4*, 569–576.

Streeter, J. and De Taboada, L. (2012). Low level light therapy for enhancement of neurologic function. U.S. Patent Application 11/844,205. Filing date August 23, 2012.

Sutherland, J.C. (2002). Biological effects of polychromatic light. Photochem. Photobiol. *76*, 164–170.

Svaasand, L.O. and Ellingsen, R. (1983). Optical properties of human brain. Photochem. Photobiol. *38*, 293–299.

Sykova, E. and Vargova, L. (2008). Extrasynaptic transmission and the diffusion parameters of the extracellular space. Neurochem. Int. *52*, 5–13.

Tang-Schomer, M.D., Johnson, V.E., Baas, P.W., Stewart, W., and Smith, D.H. (2012). Partial interruption of axonal transport due to microtubule breakage accounts for the formation of periodic varicosities after traumatic axonal injury. Exp. Neurol. *233*, 364–372.

Tascioglu, F., Kuzgun, S., Armagan, O., and Ogutler, G. (2010). Short-term effectiveness of ultrasound therapy in knee osteoarthritis. J. Int. Med. Res. *38*, 1233–1242.

Teggi, R., Bellini, C., Piccioni, L.O., Palonta, F., and Bussi, M. (2009). Transmeatal low-level laser therapy for chronic tinnitus with cochlear dysfunction. Audiol. Neurootol. *14*, 115–120.

Topakian, R., Barrick, T.R., Howe, F.A., and Markus, H.S. (2010). Blood-brain barrier permeability is increased in normal-appearing white matter in patients with lacunar stroke and leucoaraiosis. J. Neurol. Neurosurg. Psychiatry *81*, 192–197.

Toselli, R., Di Mauro, T., and Fisher, M. (2009). Endoscopic delivery of red/NIR light to the substantia nigra to treat Parkinson's disease. U.S. Patent Application 12/041,405. Filing date March 3, 2008.

Tretter, L., Takacs, K., Kövér, K., and Adam-Vizi, V. (2007). Stimulation of H(2)O(2) generation by calcium in brain mitochondria respiring on alpha-glycerophosphate. J. Neurosci. Res. *85*, 3471–3479.

Tsukahara, H., Jiang, M.Z., Ohta, N., Sato, S., Tamura, S., Hiraoka, M., Maeda, M., and Mayumi, M. (2004). Oxidative stress in neonates: evaluation using specific biomarkers. Life Sci. *75*, 933–938.

von Bussmann, K.A., Garey, L.J., and Jen, L.S. (1993). Injury-resistant retinal ganglion cells that are rich in cytochrome oxidase. Neuroreport *4*, 247–250.

Wan, S., Parrish, J.A., Anderson, R.R., and Madden, M. (1981). Transmittance of nonionizing radiation in human-tissues. Photochem. Photobiol. *34*, 679–681.

Whelan, H.T., Smits, R.L., Jr., Buchman, E.V., Whelan, N.T., Turner, S.G., Margolis, D.A., Cevenini, V., Stinson, H., Ignatius, R., Martin, T., et al. (2001). Effect of NASA light-emitting diode irradiation on wound healing. J. Clin. Laser Med. Surg. *19*, 305–314.

Whelan, H.T., Buchmann, E.V., Dhokalia, A., Kane, M.P., Whelan, N.T., Wong-Riley, M.T., Eells, J.T., Gould, L.J., Hammamieh, R., Das, R., et al. (2003). Effect of NASA light-emitting diode irradiation on molecular changes for wound healing in diabetic mice. J. Clin. Laser Med. Surg. *21*, 67–74.

Winkler, B.S. (1981). Glycolytic and oxidative metabolism in relation to retinal function. J. Gen. Physiol. *77*, 667–692.

Wong-Riley, M.T., Bai, X., Buchmann, E., and Whelan, H.T. (2001). Light-emitting diode treatment reverses the effect of TTX on cytochrome oxidase in neurons. Neuroreport *12*, 3033–3037.

Wong-Riley, M.T., Liang, H.L., Eells, J.T., Chance, B., Henry, M.M., Buchmann, E., Kane, M., and Whelan, H.T. (2005). Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome c oxidase. J. Biol. Chem. *280*, 4761–4771.

Wu, X., Dmitriev, A.E., Cardoso, M.J., Viers-Costello, A.G., Borke, R.C., Streeter, J., and Anders, J.J. (2009). 810 nm Wavelength light: an effective therapy for transected or contused rat spinal cord. Lasers Surg. Med. *41*, 36–41.

Wu, Q., Xuan, W., Ando, T., Xu, T., Huang, L., Huang, Y.Y., Dai, T., Dhital, S., Sharma, S.K., Whalen, M.J., et al. (2012). Low-level laser therapy for closed-head traumatic brain injury in mice: effect of different wavelengths. Lasers Surg. Med. *44*, 218–226.

Ying, R., Liang, H.L., Whelan, H.T., Eells, J.T., and Wong-Riley, M.T. (2008). Pretreatment with near-infrared light via light-emitting diode provides added benefit against rotenone- and MPP+-induced neurotoxicity. Brain Res. *1243*, 167–173.

Yousefi-Nooraie, R., Schonstein, E., Heidari, K., Rashidian, A., Akbari-Kamrani, M., Irani, S., Shakiba, B., Mortaz Hejri, S.A., Mortaz Hejri, S.O., and Jonaidi, A. (2008). Low level laser therapy for nonspecific low-back pain. Cochrane Database Syst. Rev. 2007, CD005107.

Yu, W., Naim, J.O., McGowan, M., Ippolito, K., and Lanzafame, R.J. (1997). Photomodulation of oxidative metabolism and electron chain enzymes in rat liver mitochondria. Photochem. Photobiol. *66*, 866–871.

Yu, D.Y., Cringle, S.J., Alder, V., and Su, E.N. (1999). Intraretinal oxygen distribution in the rat with graded systemic hyperoxia and hypercapnia. Invest. Ophthalmol. Vis. Sci. *40*, 2082–2087.

Zivin, J.A., Albers, G.W., Bornstein, N., Chippendale, T., Dahlof, B., Devlin, T., Fisher, M., Hacke, W., Holt, W., Ilic, S., et al. (2009). Effectiveness and safety of transcranial laser therapy for acute ischemic stroke. Stroke *40*, 1359–1364.