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Review

Exploring the Use of Intracranial and Extracranial (Remote) Photobiomodulation Devices in Parkinson's Disease: A Comparison of Direct and Indirect Systemic Stimulations

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Abstract. In recent times, photobiomodulation has been shown to be beneficial in animal models of Parkinson's disease, improving locomotive behavior and being neuroprotective. Early observations in people with Parkinson's disease have been positive also, with improvements in the non-motor symptoms of the disease being evident most consistently. Although the precise mechanisms behind these improvements are not clear, two have been proposed: direct stimulation, where light reaches and acts directly on the distressed neurons, and remote stimulation, where light influences cells and/or molecules that provide systemic protection, thereby acting indirectly on distressed neurons. In relation to Parkinson's disease, given that the major zone of pathology lies deep in the brain and that light from an extracranial or external photobiomodulation device would not reach these vulnerable regions, stimulating the distressed neurons directly would require intracranial delivery of light using a device implanted close to the vulnerable regions. For indirect systemic stimulation, photobiomodulation could be applied to either the head and scalp, using a transcranial helmet, or to a more remote body part (e.g., abdomen, leg). In this review, we discuss the evidence for both the direct and indirect neuroprotective effects of photobiomodulation in Parkinson's disease and propose that both types of treatment modality, when working together using both intracranial and extracranial devices, provide the best therapeutic option.

Keywords: Animal models, behavior, mitochondrial activity, neuroprotection, neurotrophic factors

INTRODUCTION

Many previous studies have reported that photobiomodulation improves locomotion and is neuroprotective in animal models of Parkinson's disease, as well as improving motor signs and, in particular,

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non-motor symptoms in people with Parkinson's disease. The precise mechanisms behind these benefits are not clear, but two have been suggested. First, direct stimulation, with photobiomodulation acting directly on the distressed neurons and second, remote indirect stimulation, with photobiomodulation influencing circulating cells and/or molecules and then these acting on these neurons. In the sections that follow, we will consider first, the clinical syndrome and pathophysiology of Parkinson's disease, followed by the evidence for both direct and indirect systemic stimulations in animal models and in people with Parkinson's disease. We will then propose that both types of stimulation, when used together, will offer the most benefit to people with Parkinson's disease.

PARKINSON'S DISEASE

Parkinson's disease, first described as the "paralysis agitans" or "shaking palsy" by James Parkinson over two hundred years ago, has now been estimated to affect more than ten million people worldwide. The incidence of the disease increases with age, with only about 4% of people with Parkinson's disease being diagnosed before age 50. Overall, it is estimated that Parkinson's disease affects 1% of the global population over the age of 60.

Clinical syndrome

Parkinson's disease is characterized by distinct cardinal motor signs, including akinesia and/or bradykinesia, lead-pipe rigidity, resting tremor, and postural instability. Initial diagnosis is made when an individual shows any two of these cardinal signs, with at least one of the two being tremor or bradykinesia, as well as a positive response to dopaminergic drug therapy [1–5]. In addition to these motor signs, there are a number of non-motor symptoms, including apathy, cognitive impairment, depression, anxiety, fatigue, anosmia, sleep disorders, anhedonia, and gastrointestinal and autonomic dysfunction [5–7]. The classic pre-motor features of anosmia/hyposmia and rapid eye movement sleep behavior disorder with dream enactment may precede the recognizable motor signs by several years [1–5].

Pathology

A striking feature of Parkinson's disease is that the main zones of pathology are rather discrete, within distinct neuronal groups lying mainly within the brainstem, deep in the brain. The main zone of pathology is within substantia nigra pars compacta (SNc) of the midbrain. These neurons, most of which are dopaminergic, undergo a progressive degeneration over a period of many years. In addition, there are losses in other localized regions; for example, other dopaminergic neurons in the midbrain and olfactory bulb, the noradrenergic neurons of the locus coeruleus, the cholinergic neurons of the pedunculopontine tegmental nucleus, the serotonergic neurons of the raphe nuclei, together with neurons of the dorsal motor nucleus of the vagus nerve. At later stages, there is some neurodegeneration across the cortex also [1, 3, 5, 8, 9].

Mechanisms of degeneration

The mechanisms that lead to the death of neurons, particularly the dopaminergic ones, have come under much scrutiny in recent years. There is general agreement that—regardless of the initial trigger, whether it be an environmental toxin or genetic mutation—these mechanisms are apoptotic, involving a slow breakdown of cellular constituents, rather than necrotic, which is associated with a more rapid breakdown of cellular constituents [10]. This apoptotic process has two major, not necessarily mutually exclusive, mechanisms. These are mitochondrial dysfunction and Lewy body accumulation [11].

The mitochondria are the engine rooms of neurons; they produce the energy (ATP) that fuels so many intrinsic cellular pathways and generate factors reducing the oxidative stress of neurons. After parkinsonian insult, there is a progressive accumulation of mutations in mitochondrial DNA impairing efficient mitochondrial function. This process leads to an increase in the levels of reactive oxygen species, generating oxidative stress, leading subsequently to neuronal death [12]. Some of the key evidence for mitochondrial dysfunction in Parkinson's disease comes from the discovery that many experimental toxins used to generate animal models, such as 6OHDA (6 hydroxydopamine) or MPTP (methyl-4-phenyl-1,2,3,6-tetrahydropyridine), target the mitochondria and cause extensive oxidative stress and damage [13]. Further, many of the gene mutations associated with the disease, for example PINK1, parkin, SNCA and LRRK2, have been linked to mitochondrial dysfunction and neuronal death [14–16]. Finally, low levels of mitochondrial complex I—the largest enzyme complex in the electron transport chain driving ATP production—have been reported in people with Parkinson's disease [17].

Lewy bodies, that are found within the dopaminergic neurons in the SNc of people with Parkinson's disease, are made up mainly of abnormal aggregations of α-synuclein. These aggregations are considered toxic to the neurons [18, 19]. Under normal circumstances, there are low levels of α-synuclein within the mitochondria, but when factors unknown stimulate an increase in accumulation, this leads to mitochondrial complex I deficits, oxidative stress, and neuronal death [20].

Gliosis and growth factors

Parkinson's disease is associated also with an increase in glial cell number or a gliosis [21]. This gliosis does not appear to be the initial trigger for disease onset but is essential to the ongoing pathology. The process has traditionally been interpreted as toxic to neurons, for example by inhibiting axonal regeneration by forming glial scars and/or secreting pro-inflammatory cytokines. More recently, however, it has been associated with beneficial effects, with the release of growth factors such as glial derived neurotrophic factor (GDNF). Indeed, in animal models of the disease, many authors have reported an increase in GDNF expression in the basal ganglia, presumably relating to a repair and regrowth of dopaminergic axons and terminations striatum [22–25]. Unfortunately, this increase in GDNF is not long lasting and levels revert to normal within a short-period after insult (e.g., weeks). In people with Parkinson's disease, there is evidence of a reduction in GDNF levels across the basal ganglia [26], of which, has been linked to the degeneration of the dopaminergic neurons [24].

Vascular dysfunction and the blood-brain barrier breakdown

There are also indications that vascular dysfunction contributes to the pathogenesis of Parkinson's disease. The degeneration of dopaminergic neurons may be triggered and/or fueled after endothelial cell damage and a compromise of blood-brain barrier function [27–29]. The degenerative vascular morphology seen in Parkinson's disease includes the formation of endothelial cell clusters, that are presumed to contribute to the fragmentation of capillaries and a breakdown of the entire capillary network nourishing the neurons [29]. In this context, the toxins that induce parkinsonism in animal models, namely 6OH DA and MPTP [13], have been shown to generate substantial disruption of the blood-brain barrier, suggesting that at least part of their toxic effect on neurons is by compromising the efficacy of the vascular system [28, 30].

Abnormal circuitry

Taken all together, the loss of midbrain dopaminergic neurons and subsequent reduction of striatal dopamine levels, generates a cascade of abnormal circuitry across the brain. For motor signs, the subthalamic nucleus of the basal ganglia is central. With the loss of dopamine, this small nucleus becomes overactive, leading to less overall motor activity and abnormal oscillations in the thalamus and cortex [1, 8, 31]. For non-motor symptoms, a number of mechanisms have been suggested for many of them. For example, anosmia has been linked to the presence of Lewy bodies and neuronal loss in the olfactory centers, while constipation appears to involve the dysfunction and presence α-synuclein aggregates within the enteric nervous system, together with the dorsal motor nucleus of the vagus. Further, depression has been associated with the neuronal loss in the locus coeruleus and raphe nuclei and cognitive impairment is linked to neuronal loss and α-synuclein aggregates across the cortex [3, 5, 32].

Treatments

As for current treatments, with the onset of the first motor signs of the disease and diagnosis, people with Parkinson's disease are treated with dopamine replacement drug therapy, that aims to replace the dopamine lost from the system. L-Dopa, converted to dopamine in the brain, is often first-line therapy and is typically highly efficacious at reducing motor signs. Dopaminergic treatment of people with Parkinson's disease becomes more difficult later in the disease and side-effects such as drug-induced dyskinesias and neuropsychiatric disturbances, for example visual hallucinations, may develop [1–3, 5]. As the disease progresses, some people with Parkinson's disease may be candidates for deep brain stimulation at high frequency, most commonly targeting the subthalamic nucleus [33]. This surgery serves to correct and/or adjust the abnormal activity of the basal ganglia generated by the loss of dopamine from the system [33]. As with the dopamine replacement drug therapy, deep brain stimulation has been shown very effective in treating the motor signs of the disease [33]. The non-motor symptoms add substantially to

a loss in the quality of life in people with Parkinson's disease and treatment for these is problematic; the non-motor symptoms are less responsive to dopamine drug therapy and to deep brain stimulation.

A key feature of the current mainstay treatments—dopamine replacement drug therapy and deep brain stimulation—is that they are largely symptomatic rather than disease-modifying or neuroprotective. In both cases, they serve to enhance the functionality of the neuron rather than promote its survival. As it stands, there is no current effective neuroprotective treatment option available for people with Parkinson's disease, one that reliably stops or even slows the course of the disease. The bulk of more recent studies attempting to develop a neuroprotective treatment have targeted the mitochondrial dysfunction, focusing on helping these organelles resume normal activity.

PHOTOBIOMODULATION

Previous studies have indicated that many, if not all, of the benefits reported by photobiomodulation, the application of red to infrared light ($\lambda = 600$–1070 nm) on body tissues, are through its influence on mitochondrial activity. As a consequence, and given that mitochondrial dysfunction is so central to the pathogenesis of Parkinson's disease, photobiomodulation has been explored by many authors as a potential therapeutic treatment for this, as well as other neurodegenerative disorders [34–37]. Although the precise mechanisms on how photobiomodulation may stimulate mitochondrial activity in distressed neurons and be neuroprotective are not entirely clear, two general mechanisms have been proposed (Fig. 1); 1) direct stimulation of the distressed neurons and 2) indirect stimulation in which an intermediary transduces protection to the distressed neurons [35, 36]. In the sections that follow, these different types of stimulations will be discussed. First, issues regarding safety and dosage will be considered briefly.

Safety and dosage

There are few, if any reports of photobiomodulation having a detrimental, toxic effect on body tissue, nor of it having any side effects [34, 36]. The total light energy required to elicit a therapeutic effect is generally < 10 J/cm^2, although this varies greatly in the literature, ranging between 1–60 J/cm^2 [34, 36]. In many models of disease and systems, it has been reported that light applied in pulses or short bursts is more effective than if applied continuously [34, 36]. Further, there is a biphasic dose response for light, in that it is most effective at intermediate doses, but not at very low or extremely high doses, the so-called hormetic effect. There may be a threshold, with cells requiring a set level of light energy to gain benefit,

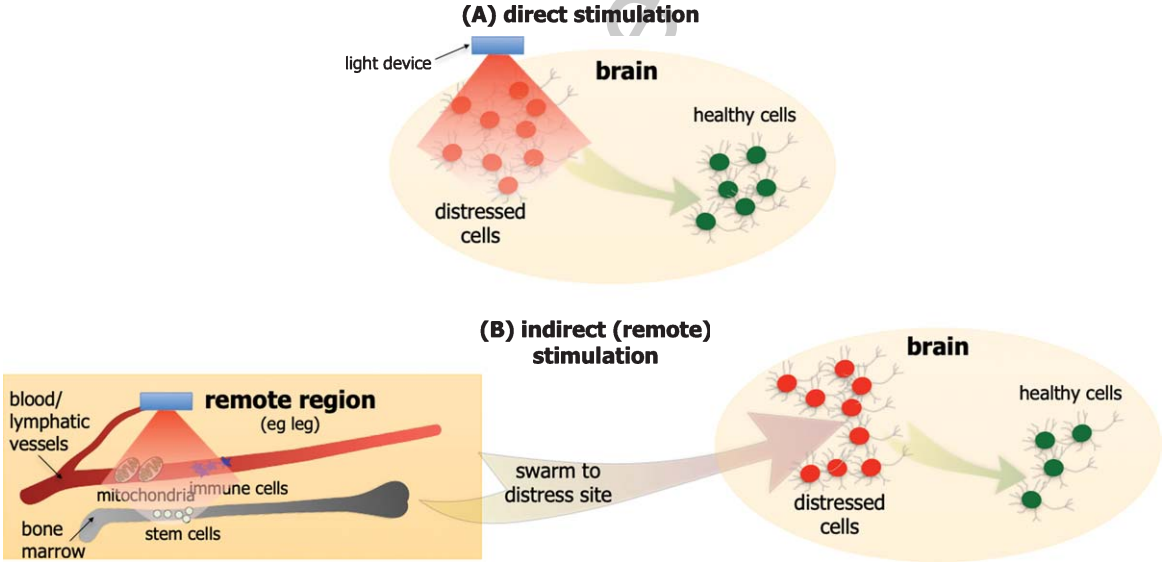

Fig. 1. Schematic diagram of the impact of (A) direct and (B) indirect (remote) photobiomodulation stimulation. When photobiomodulation is applied directly to distressed cells (red cells; e.g., within brain) it triggers intrinsic cellular mechanisms that help survival and function (i.e., healthy cells, green). When photobiomodulation is applied indirectly, to a remote and distant organ (e.g., leg), then it may activate circulatory cells and/or molecules that swarm to distressed cells in another organ (e.g., brain) and helps survival and function. Although both stimulations have been shown to be effective in animal models, the direct stimulation is the more effective.

but after that level is reached, the effects subside [34, 36].

Direct stimulation

This stimulation relies on photobiomodulation being applied directly on the distressed neurons (Fig. 1). The photons stimulate chemical changes within neurons directly, with light energy being converted to metabolic energy with a subsequent influence on neuronal function and survival.

Mechanisms

The first step involves light being absorbed by a photoacceptor and the best known one is cytochrome c oxidase, unit IV in the mitochondrial electron transport chain. Cytochrome c oxidase has two heme and two copper centers that absorb light within two bands across the red to near infrared range (600–700 nm and 760–940 nm). The mechanism involves light dissociating nitric oxide from its heme and copper binding sites in the cytochrome c oxidase, thereby allowing the binding of oxygen. Electrons are then transported along the respiratory chain and a translocation of protons across the mitochondrial membrane occurs. This produces a proton gradient across the membrane, one that drives a rotatory motor called ATP synthase, the enzyme that makes ATP. The net result is an increase in the mitochondrial membrane potential and a surge of ATP energy. The released nitric oxide, in addition to allowing oxygen binding, triggers the vasodilation of nearby blood vessels, increasing blood (and lymphatic) flow. With activation of cytochrome c oxidase, small amounts of reactive oxygen species are released (within normal levels), that then activate transcription factors in the nucleus of the neuron [34]. It should be noted that in a recent study using mouse and human cell lines lacking cytochrome c oxidase, photobiomodulation was nevertheless shown to increase in ATP levels, indicating that there must be other photoacceptor(s) within the neurons [38].

Water within the mitochondria has been suggested to be one such "other" photoacceptor [39, 40]. Layers of nanowater are found within the folded membranes of the mitochondria and these tend to get viscous. This increase in water viscosity impedes ATP synthase and hence the production of ATP, leading ultimately to distress in the neuron. Photobiomodulation has been related to a decrease in the viscosity of the water, leading to an increase in the efficiency of ATP synthase, higher levels of ATP, and lower levels of reactive oxygen species [39, 40].

There is also evidence that chlorophyll metabolites may act as photoacceptors [41]. When incubated with a light-capturing metabolite of chlorophyll, mitochondria were found to have higher levels of ATP after photobiomodulation. Further, when rodents were fed a chlorophyll-rich diet, the chlorophyll metabolites were found concentrated within the mitochondria. The chlorophyll ingested by animals appeared to be converted into a variety of metabolites that become incorporated within mitochondria across a number of body tissues. These metabolites, when treated with photobiomodulation, can catalyze the reduction of coenzyme Q, leading subsequently to cytochrome c oxidase activation and an increase in mitochondrial activity and ATP production.

The photobiomodulation-induced increase in mitochondrial activity in the distressed neurons leads to the expression of various protective genes, most notably genes encoding neurotrophic factors. These neurotrophic factors may then stimulate neurogenesis and synaptogenesis across the brain. Photobiomodulation has been reported to increase the proliferation of neuroprogenitor cells, the formation of new synapses and the expression of the BDNF (brain-derived neurotrophic factor) in the hippocampus of an animal model of traumatic brain injury [42] and GDNF in the striatum of an animal model of Parkinson's disease [25]. Similar findings of photobiomodulation-induced increases in neuroprogenitor cell proliferation in the subventricular zone have been made in a rat model of stroke [43].

Taken all together, photobiomodulation appears to stimulate intrinsic self-protective mechanisms that help distressed neurons protect and repair themselves from any insult or damage. These photobiomodulation-induced intrinsic mechanisms prompt an increase in energy production for the neuron, together with stimulating the expression of genes and growth factors involved in improving their survival. Further, photobiomodulation increases the local blood (and lymphatic) flow which helps in the perfusion of the region [34]. These all contribute to a healthier and more resilient neuron, in a better position to protect and to repair itself from insult and/or to maintain its ongoing survival and homeostasis [34, 44].

Applications in animal models of Parkinson's disease: Neuroprotection and behavioral changes

Over the last fifteen years or so, an impressive body of evidence for photobiomodulation being a disease-modifying or neuroprotective agent in a range

of animal models of Parkinson's disease, from flies (drosophila) to monkeys, has accumulated [36, 45]. Many studies have reported that, in both toxin-induced and transgenic models, photobiomodulation increases the survival of dopaminergic neurons in the SNc and their striatal terminations, together with reducing the gliosis and increasing GDNF expression. The following neuroprotective effects of photobiomodulation have been based on direct stimulation, with photobiomodulation reaching and acting directly on the distressed neurons.

The first evidence for photobiomodulation being neuroprotective using a Parkinson's disease model were *in vitro* [46, 47]. These studies showed that photobiomodulation reduced cell death, increased ATP and decreased oxidative stress in neurons exposed to parkinsonian toxins. In cultures of human neuroblastoma neurons engineered to overexpress α-synuclein, photobiomodulation was also reported to increase mitochondrial function and reduce oxidative stress after toxin exposure [48]. In addition, in hybrid neurons bearing mitochondrial DNA from people with Parkinson's disease, mitochondrial movement along axons improved considerably after photobiomodulation [48]. Photobiomodulation has also been shown to rescue major mitochondrial defects in drosophila pink1 mutants and mouse dopaminergic neurons [49].

Following on from these pioneering *in vitro* studies, the first series of *in vivo* studies were on the MP TP-treated mouse model of Parkinson's disease. In MPTP-treated mice [50–57], photobiomodulation protected many dopaminergic neurons from toxic insult and subsequent degeneration. Further, results were similar whether photobiomodulation was applied before, at the same time or well after the insult, indicating that photobiomodulation both conditioned healthy neurons to resist a subsequent insult and rescued damaged neurons following an insult [51, 55]. The rescue of neurons is particularly relevant to the clinical reality of the parkinsonian condition, in which individuals have, at presentation, already suffered significant degeneration, so that treatment follows neuronal loss. A neuroprotective effect after photobiomodulation has also been examined in two transgenic rodent models. In the K369I tau transgenic model, which manifests a progressive degeneration of dopaminergic neurons in the SNc over a period of five to six months, photobiomodulation decreased oxidative stress and hyperphosphorylated tau, as well as increased dopaminergic neuronal survival in the SNc [58]. In an α-synuclein rat model, photobiomodulation-treated animals had more dopaminergic neurons in the SNc and terminations in the striatum compared to the untreated animals [59].

The application of photobiomodulation in the experiments described above was transcranial, using a hand-held device with light directed at the animal's head. In mice, the distance between the cranial surface and the SNc is in the vicinity of 5 mm. Hence, photobiomodulation applied in this way can reach the SNc and offer direct stimulation. In the larger primate brain, however, where the distance between cranial surface and SNc is greater, being 40–50 mm in monkeys and 80–100 mm in human, transcranial application of photobiomodulation would not reach the distressed SNc neurons directly [36]. Hence, in order to offer direct stimulation in the primate brain, an intracranial optical fiber device delivering 670 nm light was developed. The feasibility of this device was tested initially in MPTP-treated mice, with implants into the lateral ventricles [60], and in 6OHDA-lesioned rats, with implants into a midline region of the midbrain [61]. In both cases, photobiomodulation was not toxic to the surrounding tissue, even though the photobiomodulation source lay directly on neural tissue, nor did it generate any behavioral deficits; in fact, neuroprotection of dopaminergic neurons in the SNc was evident with this intracranial device, similar in magnitude achieved transcranially.

With these findings that the intracranial device was well-tolerated by rodents, the intracranial device was developed for use in the monkey brain, with a clear view for it to be developed even further for clinical use in people with Parkinson's disease. Using the MPTP-treated model, it was found that all of the photobiomodulation-treated MPTP monkeys had a greater number of surviving dopaminergic neurons in the SNc compared to those that were untreated [62]. In addition, the density of dopaminergic terminations in the striatum was greater in the photobiomodulation-treated animals compared to those that were not treated [62].

Photobiomodulation not only had a positive effect on the survival of the distressed neurons in Parkinson's disease, but it also had an impact on the resident glial cells. Previous studies have shown that photobiomodulation influenced the MPTP-induced gliosis in the basal ganglia of mice [53, 57] and monkeys [63]. In addition, in a lipopolysaccharide rat model, photobiomodulation was shown to reduce dopaminergic neuronal degeneration and gliosis within the SNc [64]. It is not clear if the photobiomodulation-induced reduction in gliosis is due to a direct action

on the glial cells or secondary to the survival of the neurons. If acting directly on the glial cells, the photobiomodulation could stimulate a neuroprotective role for these cells, perhaps by triggering various intrinsic cellular mechanisms, resulting in an increase of their secretion of anti-inflammatory agents and a decrease of their pro-inflammatory ones [21]. This in turn, would result in a greater survival of dopaminergic neurons in the SNc and their terminations in the striatum [63].

Photobiomodulation has been shown also to influence the expression of GDNF in the striatum of a MPTP-treated monkey model of Parkinson's disease [25]. This expression has been suggested to help damaged dopaminergic afferents regrow and establish new synaptic contacts and second, to switch-on the dopaminergic phenotype (i.e., tyrosine hydroxylase expression) in many striatal cells, presumably helping to restore dopamine levels in the striatum after MPTP insult [25].

In addition, photobiomodulation appears to enhance blood-brain barrier integrity and reduce cerebrovascular leakage. In MPTP-injected mice, that show profound vascular leakage in the midbrain and caudate-putamen complex, daily transcranial photobiomodulation with 670 nm light significantly mitigated vascular leakage in both brain regions to near control levels [30].

Many previous studies, in a range of animal models of Parkinson's disease, have reported that there are locomotive behavioral changes that accompany the photobiomodulation-induced neuroprotection, that this neuroprotection is indeed useful at a functional level. In the MPTP-treated mouse model, behavioral tests have shown that photobiomodulation improved locomotion [52, 54–56]. In particular, when mice were photobiomodulation-treated either before or at the same time as the MPTP insult, their locomotive deficits were reduced and their activity returned to control levels well before those in the MPTP group [55]. From a post-treatment of photobiomodulation series, when mice were photobiomodulation-treated well after the MPTP insult, their behavioral deficits dissipated almost immediately, within minutes after treatment (see also [51]).

Photobiomodulation has also been shown to improve behavior in a 6OHDA-lesioned hemi-parkinsonian rat model of the disease, where there was a markedly reduced apomorphine-induced rotational behavior [61]. There is also evidence in a drosophila model, where photobiomodulation rescued flight defects in PINK1 mutants [49].

In the MPTP-treated monkey model, where the animals develop clear human-like signs of the disease that could be assessed clinically, the MPTP-treated monkeys exposed to photobiomodulation had a much lower mean clinical score than the MPTP-treated monkeys that were not exposed. In addition, the overall locomotive movement of the MPTP-treated monkeys exposed to photobiomodulation was greater than those that were not exposed [62]. These improvements in clinical signs and movement in the photobiomodulation-exposed MPTP-treated monkeys were still evident up to three weeks after the short, five-day period of photobiomodulation, indicating that the therapeutic effects are long-lasting and not confined to the periods of treatment application [62].

In summary, there is a wealth of experimental evidence for neuroprotection by photobiomodulation in a wide range of animal models of Parkinson's disease, from toxin-induced mouse, rat and monkey models to transgenic drosophila, mouse and rat models. These findings assume greater importance when considering that currently, there is no effective neuroprotective treatment option for people with Parkinson's disease. Further, there is clear evidence that photobiomodulation had an impact on locomotive behavior in a number of animal models of Parkinson's disease, from drosophila to rodents to monkeys; in the monkey model, there was also a clear reduction in the clinical signs of the disease, many of which are apparent in people with Parkinson's disease. In each of these experimental studies, the photobiomodulation-induced behavioral improvements and neuroprotection were based largely on the notion of direct stimulation, that the light reached and acted directly on the distressed neurons.

Indirect systemic stimulation: Extracranial (remote) application

Despite the profound neuroprotective effects of direct stimulation in animal models, a major barrier to a practical, clinical translation of this approach for people with Parkinson's disease is the delivery of light energy to the vulnerable midbrain. Only 1–3% of light energy penetrates the skin and skull, with less than 1% of that light energy penetrating 12 mm of brain tissue (reviewed by [34, 36, 65]). While the intracranial mode of delivery attempts to circumvent this inherent barrier, it is highly unlikely that the more common approach of transcranial photobiomodulation achieves sufficient penetration of light energy to

directly stimulate the parts of the brain first affected in Parkinson's disease [35, 36, 60].

Nonetheless, an increasing number of studies are reporting that photobiomodulation has indirect, systemic, protective effects that can be harnessed to overcome practical barriers to implementation. Structures and systems such as blood vessels, lymphatics, bone marrow, and the gut microbiome (see below) can all be accessed far more readily by light applied externally [35, 36]. For example, light penetration across mouse abdominal skin has been measured at 20–30% of emitted intensity (Johnstone, unpublished data); this drops to 15% when fur is intact [66]. Further, it has been shown that light can penetrate across a number of body parts in both human living subjects and cadavers, up to 50 mm thickness [67]. This phenomenon is somewhat analogous to the "abscopal effect" sometimes observed following radiation treatment of metastatic cancer [53, 68], and to the well-established intervention of remote ischemic conditioning. Given the apparent similarity to remote ischemic conditioning, we coined the term "remote photobiomodulation" to describe treatment modalities that take advantage of the indirect systemic effects of photobiomodulation by targeting light at a distal tissue with the purpose of providing protection to a non-irradiated tissue (e.g., the brain; Fig. 1) [69].

In the first comprehensive demonstration of this phenomenon, Rochkind and colleagues showed in rats that photobiomodulation directed at a lesion on one side of the body can enhance healing bilaterally, in the context of cutaneous wound, burn injury, and nerve injury [70]. A number of supporting studies have since followed, as reviewed elsewhere [71]. Others have attempted to define the mechanisms, with a focus on bone marrow-derived stem cells as the protective mediator, showing that photobiomodulation of the bone marrow in a rat model of myocardial infarction leads to a greater reduction in infarct size and ventricular dilatation than when photobiomodulation is targeted directly at the heart infarct [72]. The benefits of remote photobiomodulation targeting the bone marrow of the tibia have been subsequently demonstrated in a rat model of ischemia-reperfusion kidney injury [73].

Mounting evidence suggests that remote photobiomodulation-induced protection extends to the brain (Fig. 1). In the MPTP-treated mouse model, irradiating the dorsum of the animals with 670 nm following MPTP injection, while simultaneously shielding the head with aluminum foil, yielded substantial neuroprotection; MPTP-treated mice with photobiomodulation of the body had more dopaminergic neurons than sham-treated MPTP mice [53, 66, 74]. In addition to mitigating damage following an insult, a subsequent study demonstrated that remote photobiomodulation provided neuroprotection when administered as a pre-conditioning intervention. For example, pre-treating the body of mice with 670 nm light protected them from subsequent MPTP intoxication; mice receiving remote photobiomodulation showed less MPTP-induced dopaminergic neuronal loss and less abnormal neuronal activity in the caudate-putamen complex, as assessed by Fos immunohistochemistry [66].

In addition to providing neuroprotection to animal models of Parkinson's disease, remote photobiomodulation has also shown efficacy in other disease models. For example, Farfara and colleagues demonstrated that photobiomodulation of the tibia improved memory performance and reduced hippocampal amyloid-β burden in the 5xFAD transgenic mouse model of Alzheimer's disease [75], while Saliba and colleagues found that daily remote photobiomodulation improved visual function in a mouse model of streptozotocin-induced diabetic retinopathy [76].

The mechanisms underpinning remote photobiomodulation-induced neuroprotection remain unclear at this time. Some potential mediators that have been proposed include stem cells (particularly mesenchymal stem cells), immune cells, cytokines and chemokines, mitokines, and the microbiome, as reviewed elsewhere [71]. For example, photobiomodulation of the abdomen has been shown to modify microbial diversity in the gut in a potentially beneficial way, increasing the population of specific bacteria that are associated with a healthy gut microbiome [77]. This may be particularly relevant for Parkinson's disease given the mounting evidence that the gut-brain axis might be central to disease pathogenesis [5]. Additionally, a recent discovery that cells can secrete intact mitochondria and that functional mitochondria can be detected in blood [78] gives rise to the intriguing possibility that photobiomodulation may modify the activity of circulating mitochondria which in turn transduce protective effects to remote tissues such as the brain.

The discovery that photobiomodulation has indirect systemic effects might partly explain the reported benefits of transcranial photobiomodulation in people with Parkinson's disease (see below). It is possible that the absorption of light by the scalp, skull, and superficial layers of the brain triggers mechanisms

that confer protection to remote, non-irradiated deeper regions of the brain. While this remains to be demonstrated empirically and mechanisms have not been explored, it has been recently discovered that bone marrow cells in the skull migrate into the brain following acute injury, and that these cells transit directly through microscopic vascular channels crossing the skull cortex [79]. It is possible that, as for the tibia, photobiomodulation targeted at the skull mobilizes stem cells in the bone marrow that are recruited directly to sites of vulnerability or damage, where they release neurotrophic factors that provide neuroprotection.

Overall effect on people with Parkinson's disease using transcranial devices

In early 2016 clinical observations of people with Parkinson's disease using home-made transcranial photobiomodulation devices began in Tasmania, Australia [80–82]. Patients and clinicians were focused on changes in motor signs, and indeed motor improvements were seen, particularly tremor, gait, fine finger control, writing, and facial animation. Patients and carers then began observing other changes, especially improvements in sleep quality, energy levels, re-kindling of interest in previously neglected activities, cognitive function, increased social engagement and self-confidence as well as improvements in olfaction, anxiety, and depression. People with Parkinson's disease described the return of "the capacity for joy", as well as a return of a sense of self, "I've got my personality back" and "I feel like me again" [80–82]. Spouses provided valuable insights in this regard, most often it was the wife of the person with Parkinson's disease who first observed the positive changes in her husband.

The animal models of Parkinson's disease do not provide evidence for photobiomodulation influencing non-motor symptoms of Parkinson's disease so there was no basis to anticipate such changes. Without perhaps being aware of it, patients, carers, and clinicians were comparing the effect of photobiomodulation with the effect of dopaminergic medication, hence the focus on motor signs. This is also the focus of a recent randomized control trial of photobiomodulation in people with Parkinson's disease in which gait speed was found to improve [83].

Non-motor symptoms, especially fatigue, apathy, and sleep disturbance are notoriously difficult to treat and yet they have a profound impact on patient quality of life and carer burden. Fatigue often predates the cardinal motor signs in Parkinson's disease, and while early use of dopaminergic medication can give some relief, fatigue is an all too common part of the disease process [84]. Apathy is a common, subtle and debilitating symptom in Parkinson's disease, and has been found to be associated with more severe motor signs, higher depression scores, and reduced cognitive function [85]. When present, apathy is considered irreversible. Sleep disturbance is highly prevalent in Parkinson's disease, and its presence reduces patient quality of life and increases carer burden [86]. The consistent reports of improvement in energy, motivation, and sleep quality following daily transcranial photobiomodulation in people with Parkinson's disease are of major clinical importance. Encouragingly, improvements in fatigue, apathy and sleep disturbances have been maintained for up to four years (Hamilton and Nicklason, personal observations).

Low mood and anxiety are common in Parkinson's disease. Pharmaceutical treatments are available but are not always helpful, partly because of side effects [5]. The case reports indicate that mood, anxiety, and the capacity to cope with previously anxiety-provoking situations are improved by transcranial photobiomodulation. Concentration, attention, memory, and decision-making are part of the reduction of cognitive function seen in Parkinson's disease, a source of fear for patients and carers and a major factor in the increasing Parkinson's-related health care costs [87]. That cognitive function can improve in people with Parkinson's disease using transcranial photobiomodulation is of importance at the individual and societal level.

The reports of other non-motor symptom changes are exciting. Improvements in olfaction have been reported by people with Parkinson's disease using transcranial photobiomodulation. Self-assessment of olfaction is not a reliable marker, as patients can both over- and underestimate their sense of smell, but the consistency of case reports suggests the potential for olfactory improvement. Reports of improvements in social engagement, the return of the capacity to experience joy, exhilaration, and feeling "like me" again are intriguing and important. Equally important is the finding that all reported improvements from transcranial photobiomodulation were achieved without adverse side effects. As well, daily use of transcranial photobiomodulation has a very high degree of compliance [80–82]. That this novel treatment modality is well accepted, safe and associated with positive changes in many non-motor symptoms indicate that transcranial photobiomodulation has potential to

improve patient quality of life, reduce carer burden and reduce the burgeoning health care costs in Parkinson's disease management [87].

The mechanisms that generate these improvements in non-motor symptoms after transcranial photobiomodulation are not clear, although it is tempting to speculate. Given the wide-ranging set of symptoms—for example, fatigue, apathy, sleep disturbance, mood, anxiety, anosmia, confidence, and cognition, all associated with distinct functional areas of the brain—together with the fact that the transcranially applied photobiomodulation can only penetrate 20–30 mm through body tissues, it is likely that there was an activation of the different areas of the cerebral cortex associated with these functions. The cortex lies within ∼10 mm of the cranial surface, well within reaching distance of transcranially applied photobiomodulation. Indeed, transcranial photobiomodulation has been shown to influence cortical activity substantially [88–94]. Many of the non-motor symptoms, from apathy to sleep disturbance and from mood to cognition, may have been improved after activation of different regions of the prefrontal cortex. Olfaction may have been improved by stimulation of the posterior orbitofrontal cortex.

The activation of different cortical areas is not necessarily disease-modifying or neuroprotective; it does not slow or stop the degeneration of the deep lying dopaminergic neurons in the midbrain. These diseased neurons are not within the reach of transcranial photobiomodulation [35, 36]. It is possible, however that there may be a neuroprotective aspect to this treatment through the circulation (see previous section). The focus on Parkinson's disease as a dopaminergic motor syndrome, while understandable given the long and successful history of L-dopa treatment, has the adverse effect of limiting wider consideration of Parkinson's disease. The observations of people with Parkinson's disease having improvements in non-motor symptoms support the notion that Parkinson's signs and symptoms arise from dysfunctional multi-neurotransmitter activity; it is not all about dopamine and the basal ganglia [85].

Which stimulation would work best for humans?

Over the years that followed our first report on MPTP-treated mice [50], it has become evident that the neuroprotective and improved locomotive effects gleaned from many transcranial applications of photobiomodulation, particularly in rodent models of the disease (see above), are most likely as a result of the additive combination of both direct and indirect systemic stimulations. Photobiomodulation applied in this way would reach the neurons in distress directly (given the short distances involved in rodents; ∼5 mm) but also access the vascular and immune systems in the brain and scalp to influence circulating molecules and/or cells. A key question remains of whether these stimulations can work independently of each other in people with Parkinson's disease. It is clear from previous experimental animal studies that they can. Photobiomodulation applied to cells in culture, which relies solely on direct stimulation, has been shown to be neuroprotective, indicating that indirect stimulation is not essential for neuroprotection [46–49]. On the other hand, photobiomodulation applied remotely, to a distant body part (e.g., dorsum of the animal) relying solely on indirect systemic stimulation, offers neuroprotection also, indicating that direct stimulation is not fundamental to the process [53, 66, 71–74].

So, does one type of stimulation work better than the other? From results in animal models, it has been shown that direct stimulation is more effective than the remote indirect systemic stimulation, that direct stimulation offers the better chance for distressed neurons to protect and repair themselves [53, 74] (Fig. 1). The direct stimulation may form the primary mechanism of neuroprotection, while the indirect systemic stimulation forms a secondary and complementary mechanism [35, 36].

The interplay between both types of stimulations is far from clear at present, but it is likely that each offers a particular component—each capable of working without the other—to the protection and repair of neurons. We suggest that for maximum impact, and in order to give neurons in distress the best chance of survival, that both types of stimulation should be working together. In the case of people with Parkinson's disease, this would mean using an intracranial device to offer direct stimulation, together with an external device—applied either transcranially and/or remotely to other parts of the body—offering indirect systemic stimulation.

Other neurodegenerative conditions: Could it work for Alzheimer's disease?

Photobiomodulation has been shown to be effective in a range of other neurodegenerative conditions, including the most prevalent, Alzheimer's disease. In transgenic animal models of this disease, many authors have reported improved cognitive and memory

behavior [95–97] and reduced Alzheimer-like pathologies, namely amyloid-β plaques, neurofibrillary tangles, inflammation, and oxidative stress [95–101]. The bulk of these results were from the use of an external device applied to the head of the mice, allowing for both direct and indirect systemic stimulations; photobiomodulation applied in this way can reach all zones of pathology in the mouse cortex and hippocampus directly (given short distances, < 5 mm), as well as the vascular and immune systems in the brain and across the scalp [102]. In people with Alzheimer's disease, there are also encouraging reports of improved functional connectivities across neural networks in the cortex [90], as well as improved performances in Mini-Mental State Exams and in Alzheimer's Disease Assessment Scale tests [103] after transcranial photobiomodulation (i.e., with vielight helmet). These improvements in people with Alzheimer's disease are most likely as a result of indirect systemic stimulations, as well as a partial direct stimulation; "partial" in that the extracranial device could reach the cortical zones of pathology (∼10 mm), but not the hippocampal ones located much deeper in the brain (∼80 mm from vertex, top of the cranium; ∼40 mm from temporal bone; ∼60 mm from occipital bone). Hence, for a maximum effect, we suggest that people with Alzheimer's disease use both an extracranial device, for direct stimulation of the cortical neurons in distress and indirect systemic stimulation of vascular and immune systems across brain and scalp, together with an intracranial device implanted within the hippocampus, for direct stimulation of distressed hippocampal neurons. The development of an intracranial hippocampal device is currently underway in the Benabid laboratory at Clinatec, Grenoble.

CONCLUSIONS

In many animal models of Parkinson's disease, from drosophila to monkeys, photobiomodulation improves locomotion and is neuroprotective. Such findings assume considerable relevance in that the current mainstay treatments of the disease, namely dopaminergic drug therapy and deep brain stimulation, are largely symptomatic and not neuroprotective. There are some early, encouraging observations emerging in people with Parkinson's disease as well, with improvements in motor signs and, in particular, non-motor symptoms being reported. Two mechanisms have been proposed to underpin these benefits. First, direct stimulation, with photobiomodulation acting directly on the distressed neurons and second, remote indirect stimulation, with photobiomodulation influencing cells and/or molecules that transduce protective effects to the distressed neurons. Of the two types of stimulations, the direct one appears the more effective, offering the better chance for distressed neurons to protect and repair themselves, although it is clear that the two modalities can work independently of each other. The direct stimulation may form the primary mechanism of neuroprotection, while the indirect systemic stimulation forms a secondary and complementary mechanism. We propose that for a maximal neuroprotective impact both types of stimulation should be activated, and both be working together.

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