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EC NEUROLOGY
Research Article

A Pilot Study of Photobiomodulation Therapy Using Nir: Pre and Post 810 Nm Stimulation in Patients Affected by Neurological Diseases

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Received: March 03, 2020; **Published:** March 31, 2020

Abstract

Photobiomodulation is known to become a very important tool in treatment and support of neurological disease. The cellular processes involved during Red and Infrared Light Stimulation allows alleviating and improving cognitive and motor functions. The aim of this article is to highlight the use of NIR by Cerebro® in patients with neurological disease and to assess, as it is a pilot study, the perceived improvements in everyday life. 29 Italian patients underwent NIR stimulation therapy for 1-month and were tested before and after this stimulation period in order to assess whether there will be a difference in their perception of cognitive failures that occur in everyday life based on the Cognitive Self-Assessment Questionnaire. Although the sample is small, the data collected show that there is an improvement in the perceived quality of life in each pathological group taken into account. This allows us to carry on research in this field trying to understand and improve the role of NIR during physical and neurological rehabilitation.

Keywords: *NIR; Photobiomodulation; Neurological Disease; Cerebro®; Stimulation Therapy*

Abbreviations

PBM: Photobiomodulation; LFL: Low Flow Light; nm: Nanometers; LED: Light Emitted Diode; CBF: Cerebral Blood Flow; tPBM: Transcranial Photobiomodulation; NIR: Near Infra-Red; CCO: Cytochrome C Oxidase; MMP: Mitochondrial Membrane Potential; cAMP: Cyclic Adenosine Monophosphate; ATP: Adenosine Triphosphate; ROS: Reactive Oxygen Species; NO: Nitric Oxide; AD: Alzheimer Disease; PD: Parkinson Disease

Introduction

What is photobiomodulation (PBM)?

Photobiomodulation (PBM) is an innovative way to stimulate neuronal activity and improve neurological and psychological conditions. This term describes the use of Red and Near Infrared light to relieve inflammation and pain and tissue death. The neural tissues are exposed to Low Flow Light (LFL) with wavelengths that range from 600 to 1100 nanometres (nm) depending in which therapeutic method is used [1].

Over 50 years ago, Endre Mester discovered this technique while he was working on hair growth and wound healing in mice [2]; since then PBM started being accepted in clinical practice and physiotherapy thanks to the availability of Light Emitting Diodes (LEDs) known to be safer, lower cost and better compatible with everyday life than Lasers [1].

Many preclinical studies have been conducted to evaluate safety and optimal treatment parameters of Brain PBM [3-5]. It has been shown the neuroprotective effect of NIR Light emitting diodes (LEDs) in a variety of neurological conditions such as ischemic stroke [6], Parkinson Disease [7], traumatic brain injury [8], Alzheimer disease [9] and psychological disorders such as depression and anxiety [10,11].

PBM, applied at optimum fluences (energy density) and wavelengths, is described as beneficial since it targets organs without causing aversive effects [12,13] and increases Cerebral Blood Flow (CBF) improving the brain energy metabolism [13,14]. PBM has the ability to promote neuronal protection and survival through the mediation of anti-apoptic and pro-apoptic mediators [15,16], inflammatory signalling molecules [17,18] and stimulating neurotrophic factors [5,19].

In addition to molecular benefits, PBM is demonstrated to have beneficial effects at behavioural level such as antidepressant effects, cognitive enhancement and sleep improvement [8,20-23].

The term transcranial photobiomodulation (tPBM) is used when a non-invasive light source (Laser or LED) is administrated to the brain passing through a series of layers (including scalp, periosteum, skull bone, dura mater) reaching the cortical surface [24]. Light penetration will stimulate the neurons in the outermost layer of the cortex depending on several optical parameters, anatomical and physiological factors [25].

The neurobiological and biophysical aspects are discussed in the next section in order to provide an exhaustive overview about the use of PBM in neurological disorders and clinical practice.

The mechanism underlying PBM

Photobiomodulation (PBM) involves the use of light in a non-invasive and non-thermal way. The light from the visible and near infrared spectrum provokes cellular reactions triggering neuroprotective responses, improvements in metabolism, neurogenesis, improvements in blood flow and decrease in oxidative stress and inflammation [26]. Biological tissues have different degrees of absorption and reflection of specific light wavelengths since there are different chromophores such as water, myoglobin, melanin, cytochromes, Flavin, oxyhemoglobin and deoxyhemoglobin; each one will absorb light at a specific wavelength. As an example, water molecules absorb light at wavelengths greater that 970 nm in contrast to flavins, hemoglobin and melanin that absorb light at wavelengths shorter than 600 nm. Due to these specific wavelengths range, PBM is located in the red to NIR light spectrum with several studies supporting this principle [27-29].

What about cytochrome C oxidase (CCO)?

Many are the chromophores present inside the cell making it difficult to understand the mechanism underlying the light absorption but one aspect is clear: the excitation of the mitochondrial cytochrome c oxidase (CCO) is crucial for the effect of PBM since it is a primary photo acceptor in the red to NIR light spectrum [30]. CCO is the terminal enzyme in the electron transport chain, with 13 subunits, 2 haem groups and 2 copper centres. Each of these centres can undergo oxidation or oxygenation allowing therefor 16 different oxidative states, which present different absorption levels. CCO is mostly the only molecule that has significant light absorption in the Near Infrared spectrum [31,32]. When exposed to this type of light, CCO undergoes an increase in the availability of electrons for the reduction of molecular oxygen with an increase in the mitochondrial membrane potential (MMP), increasing levels of adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP) and reactive oxygen species (ROS) which point out an improvement in mitochondrial function-

ing in cellular metabolism [26,33]. CCO enzyme activity is thought to be inhibited by Nitric Oxide (NO), especially fi the cells involved are hypoxic or damaged. This inhibitory condition may be dissociated by photons in the spectrum of the red (600 - 700 nm) and Near-infrared (760 - 949 nm) causing an increase in the mitochondrial membrane potential, more oxygen consumptions, more glucose metabolism and more ATP production [34].

What about ROS and nitric oxide?

The photons absorbed during PBM by the mitochondria increase the production of ROS; this increase triggers mitochondrial signalling pathways that lead to cytoprotective, antioxidant and anti-apoptic effects in the cell [35]. Moreover, Nitric Oxide, photo dissociated, is a vasodilator and dilator of lymphatic flow as well [36].

What about signalling mediators and transcription factors?

It is established that CCO itself is not the only photo acceptor capable of triggering cellular processes after being light- stimulated. A review by Freitas and Hamblin (2006) shows that there are no less than fourteen different transcription factors and signalling mediators activated by light exposure [26]. From the initial photon absorption to the last behavioural effect on neurological disorders, a variety of processes occur that can be beneficial for brain disorders. These processes can be divided into short-term stimulation (ATP, blood flow, lymphatic flow, cerebral oxygenations, less edema); neuroprotection (upregulation of anti-apoptic proteins, less excitotoxicity, more anti-oxidant, less inflammation); neurotrophins, neurogenesis and synaptogenesis [37].

Clinical use of PBM with NIR: A new photobiomodulation device

The aim of this paper is to describe the use of photobiomodulation with a device implemented by Cerebro®, an Italian Neuroscientific Innovative Start-up, called NIR. NIR is a helmet that is positioned on the patients head and stimulates different brain areas depending on the function that needs rehabilitation (Figure 1). The 256 LED inside the helmet allow a stimulation of 810 nm to different brain regions such as: motor, premotor and visual areas, executive, sensory and language areas.

Figure 1: *Shows the NIR helmet by Cerebro®.*

As mentioned before, such light wavelength stimulates the glucose metabolism, ATP production, oxygen consumption and improvement in mitochondrial functioning [29,32]. These triggered processes produce beneficial effects on each neurological disorder that is based on neuroinflammatory and neurometabolic deficits.

In the development of neuroinflammatory processes, three different types of cells are involved, called also "cellular players": the microglia, astrocytes and mastocytes. In the Central Nervous System, the microglia has a role in supervising the immune system; if activated, microglia promotes and sustains the inflammatory state by producing cytokine, ROS and chemokine. Mastocytes are multifunctional cells originated in the bone marrow with the ability to release the same cell mediators as the one release by microglia. Furthermore, the astrocytes have the ability to trigger functional and structural changes in pathological conditions known as astrogliosis. Therefore, these three cells are part of the same team, releasing proinflammatory cytokines and neurotrophic factors [36,37]. This is the reason why, neurodegenerative and neuroinflammatory disorders need to be considered as two sides of the same coin. Neuroinflammatory processes and microglia activation, caused by the presence of TAU protein and neurofibrillary tangles, raise oxidative stress level and cellular toxicity in Alzheimer Disease (AD). AD is also characterized by glucose hypometabolism. Several studies have been carried out that PBM stimulation in AD improves quality of life such as sleep quality and mood states as well as cognitive functions including memory and attention [38,39]. Berman., *et al.* 2017, carried out a pilot double blind, placebo controlled trial in patients with dementia to assess the effect of 28 consecutive, 6 minutes long tPBM sessions using a helmet with LED of 1060 - 1080 nm. They showed that patients improved executive functions, visual attention, immediate memory recall and working memory [40].

Cerebral Vasculopathy, another main disorder present in the majority of the elder population, is caused by the reduction or absence of blood arrival in a specific brain part and is related to the malfunction of the carotid arteries that pump blood into the brain regions. Many are the risk factors associated with cerebral Vasculopathy including the one that are unmodifiable such as advanced age, male gender and genetic familiarity. Other risk factors are smoking, no physical activity, obesity, untreated diabetes, cholesterol, hypertension and emotional events. The cerebral scenario is characterized by altered cerebral metabolism, absence of cellular nutrient (oxygen and sugar), altered blood flow and altered ability of discard waste substances [41,42]. tPBM in Cerebral Vasculopathy induces increase of CCO concentration and oxygenated hemoglobin concentration allowing the damaged cerebral region to be sustained by the oxygen and nutrient supply.

tPBM is demonstrated to have positive impact in several conditions such as stroke, traumatic brain injury and depression since the brain regions involved are mainly at a cortical level [41]. On the other hand, speaking about Parkinson Disease (PD), the impairments are located in midbrain structures with a brain depth of 90-100mm; several studies suggest that tPBM and NIR light is not able to penetrate more than 20 mm, explaining the limitation of this method [43]. However, although this assumption is correct and PBM is not able to stimulate directly the areas implicated in PD, it is clear that this modulation device is able to improve motor and cognitive functions only by stimulating the cortical areas, with benefits occurring from the second week of stimulation [44]. The cerebral scenario in PD is characterized by metabolic impairment and reduced oxidative exchanges in the blood torrent. Since neural connections become even weaker, the other process involved in PD is cell apoptosis.

In stroke patients less has been done today to determine the real benefits present after PBM stimulation; several studies, however, showed neuroprotective and neuroreparative effects of PBM therapy in chronic stroke patients [45,46]. The cerebral scenario after stroke is a neuroinflammatory phase and oxidative and vascular processes triggering cell apoptosis [46]. From a neuropsychological point of view, after PBM therapy, stroke patients experience a better sleep quality, mitigated cephalgia and a general improvement of their quality of life highlighted also by the patient's caregiver.

Our study hypothesis is that patients with different neurologic disorders would benefit of a one-month session of 810 nm cortical PBM therapy using NIR device. The improvements would be at a cognitive and behavioural level evaluated through a specific questionnaire

used to explore their perceived cognitive failures in everyday life.

Materials and Methods

Participants

Patients were recruited from two Italian Neurorehabilitation centres that they already attend for general check-ups. The patients group consisted of 29 subjects (18 females and 11 males, age range from 50 to 89, mean age 73 and SD 9,05). Among this group of patients, 19 were affected by Cerebral Vasculopathy, 5 by Alzheimer Disease and 2 by Senile Dementia; the rest of the sample is evenly divided between subjects with atherosclerosis, migraine and cognitive deficiency.

Exclusion criteria:

- Associated psychotic and psychiatric disorder
- Use of illegal substances and drugs
- Epilepsy or other seizure disorder
- Magnetic Resonance positive to neoplasm.

Inclusion criteria

- Age over 50
- Neurological Disorder in medical history
- If Dementia, established Cognitive Decline and CT MRI consistent with diagnosis
- If Cerebral Vasculopathy established CT MRI consistent with diagnosis.

Patients data divided by age and pathology are shown in the table below (Table 1 and 2).

	F	M	TOT
Alzheimer	2	3	5
Atherosclerosis	1	-	1
Cognitive Impairment	-	1	1
Dementia	2	-	2
Migraine with Aura	1	-	1
Cerebral Vasculopathy	12	7	19
TOT	18	11	29

Table 1: *Classification by pathology, in alphabetical order, and gender of the patients.*

	50 - 59	60 - 69	70 - 79	80 - 89
Alzheimer	-	-	4	1
Atherosclerosis	-	-	-	1
Cognitive Impairment	-	-	1	-
Dementia	-	-	1	1
Migraine with Aura	1	-	-	-
Cerebral Vasculopathy	3	3	8	5
TOT	4	3	14	8

Table 2: *Classification by pathology and age of the patients.*

Subject assessment

Testing included the administration of "Cognitive Self-Assessment Questionnaire (CSQ)" based on Broadbent., *et al.* 1982; Italian validation study by Stratta., *et al.* 2006 [47,48] in order to assess the individual perception of quality of life. The questionnaire consists of 25 questions regarding small errors that may occur in everyday life (forgetfulness, distractions, mental confusion, etc.). Patients are asked to answer each question and to indicate how often these errors have occurred in the past few weeks. Answers are in a 5-Likert rating scale where 1 is "very often" and 5 is "never". This questionnaire was administrated before starting the NIR stimulation and after the 10 sessions (1 month long) stimulation so that the data could be comparable as a Pre and Post assessment. Informed consent was obtained prior to initiation of treatment.

Experimental device and procedure

The experimental device called NIR by Cerebro® used 256 LED set in 4 array of 64 LEDs/array with all matched to 810 nm (Table 3 shows the NIR technical data).

Led Number	256	Power	50 mw
Output Power	15W	Total Power	15 W
Input voltage	100-240V	Optical Power	24 mw/cm^2
Input Current	0.8 (0.8a)	Work Voltage	5V --- 6A
Wavelength	810 nm	Power frequency	50 - 60 Hz

Table 3: *NIR technical data.*

Stimulation was administrated for 12 minutes twice a week for one month (Figure 2 shows the inside view of the helmet). NIR helmets allows stimulating different areas based on the impairments. For this pilot study, in order to prevent experimental bias, each patient was stimulated in each of the cortical areas that include prefrontal cortex, motor and premotor cortex, visual and linguistic areas.

Figure 2: *Shows NIR helmet and the inside position of the Near-Infrared LEDs.*

Patients were reached out for this study in November – December 2019 and retested with the questionnaire in January – February 2019 after the 1-month NIR therapy session.

At the beginning of the study, patients were seated and with the helmet on the head making correspondence between the center of the helmet and the patient's nose as to draw an imaginary line. Stimulation lasts 12 minutes.

Data analysis

Data are collected and statistically analyzed in Excel using tables and graphs according to the type of pathology and period of stimulation. Regarding the evaluation of the Cognitive Self-Assessment Questionnaire's answers, data are clustered according to the Italian validation by Stratta., *et al*. This paper classified the answers of this questionnaire into 5 main clusters so that: question number 6, 9, 11, 12, 13, 16, 17, 21, 23, 24 are part of the cluster "Memory"; question number 1, 5, 14, 15, 22, 25 are part of the cluster "Concentration"; question number 2, 3, 4 and 18 are part of the cluster " Inattention/Distractibility"; question number 8, 10 and 19 are part of the cluster "Interpersonal Intelligence"; question number 7 and 20 are part of the cluster "Memory for names".

Results and Discussion

Cognitive self-assessment questionnaire: Results

As mentioned before, the 25 questions were joined into five clusters according to the Italian version by Statta., *et al* [46]. Table 4 shows the positive ratings to all the questionnaire's answers for all 29 subjects Pre and Post NIR therapy. Test 1 is the first questionnaire administration before starting the NIR session whereas Test 2 indicates the end of the NIR therapy after one month. In each cluster, compared to Test 1, the response percentage in Test 2 is increased for each cluster.

The two histograms below (Figure 3 and 4) show how many, of the 29 patients, answered positively to the questions inside the five clusters divided for Test 1 (before starting the NIR treatment) and Test 2 (after 1-month treatment).

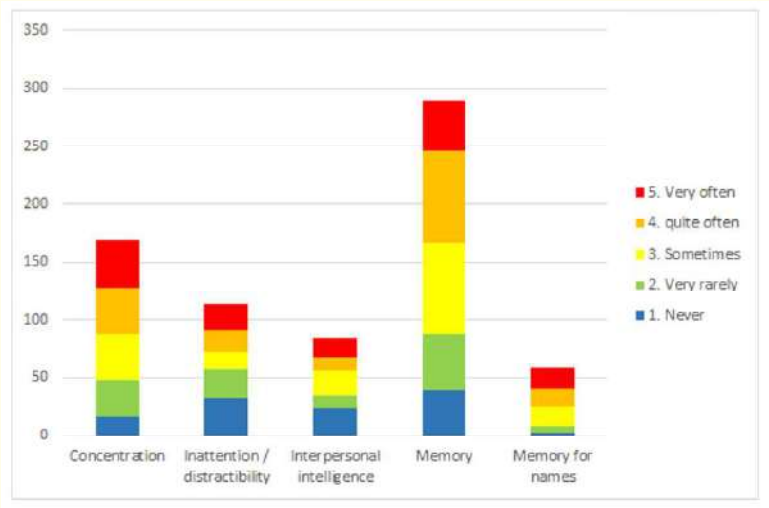

Figure 3: *Shows how many positive answers were given by the 29 patients to each question that were merged in the 5 cluster at Test 1 (before starting NIR treatment).*

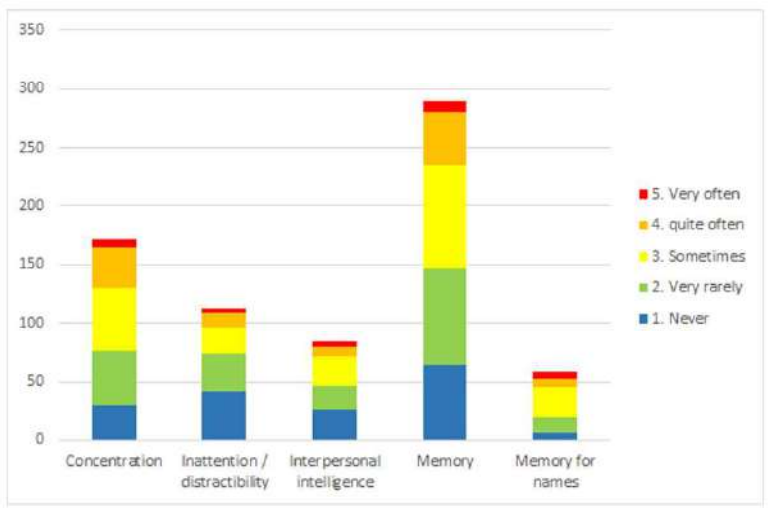

Figure 4: *Shows how many positive answers were given by the 29 patients to each question that were merged in the 5 cluster at Test 2 (after 1-month NIR treatment).*

Results according to pathology: Alzheimer disease

Since Photobiomodulation is proved to have a considerable influence on perceived cognitive improvements and quality of life, the patients group is considered and divided according to anamnestic pathology.

The Alzheimer Disease group (total number 5, 2 women and 3 men) is taken into account, considering the percentage of positive answers given to each cluster (Table 5) in the two testing periods before and after NIR stimulation and considering a mean of the answers given to each cluster in the period before NIR stimulation (Test 1) (Figure 5) and after NIR stimulation (Test2) (Figure 6).

Categories	% positive answers
Concentration	
Test 1	41%
Test 2	57%
Inattention/Distractibility	
Test 1	40%
Test 2	68%
Interpersonal intelligence	
Test 1	53%
Test 2	93%
Memory	
Test 1	32%
Test 2	66%
Memory for names	
Test 1	30%
Test 2	80%

Table 5: *Shows percentage of positive answers inside the 5 clusters by Alzheimer Disease patients before (Test 1) and after (Test 2) NIR stimulation therapy.*

Table 5 show that in Test 2, after 1-month NIR stimulation, there is a considerable difference in perceived improvement in each cluster above all in the Memory cluster.

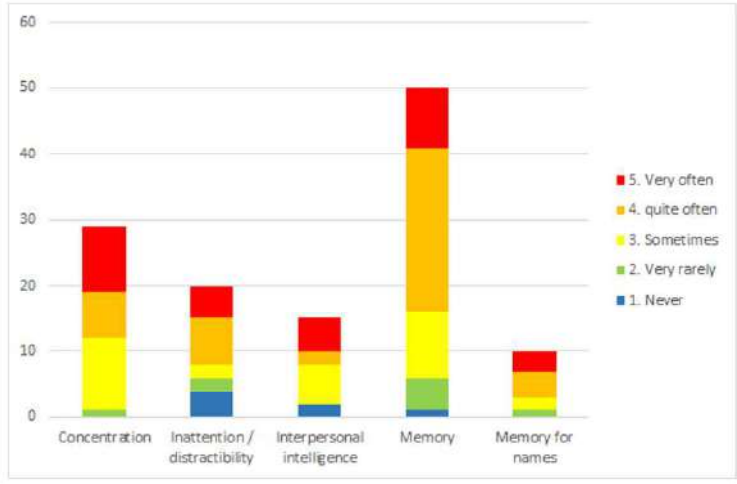

Figure 5: *Shows how many positive answers were given by the Alzheimer Disease group to each question that were merged in the 5 cluster at Test 1 (before starting NIR treatment).*

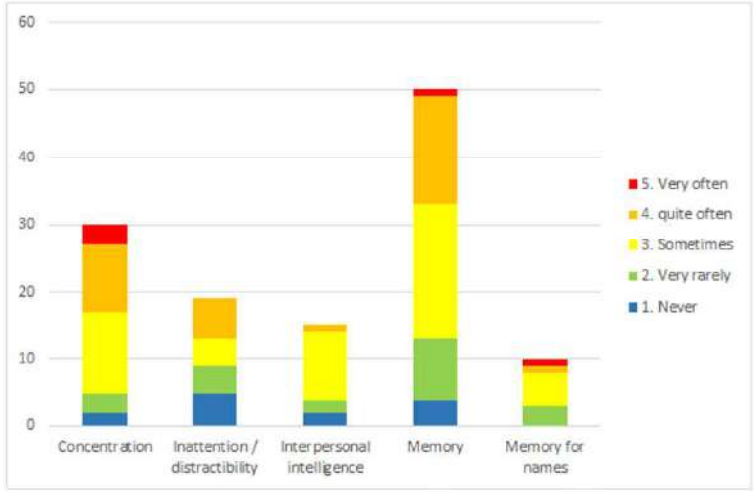

Figure 6: *Shows how many positive answers were given by the Alzheimer Disease group to each question that were merged in the 5 cluster at Test 2 (after 1-month NIR treatment).*

Results according to pathology: Cerebral vasculopathy

The Cerebral Vasculopathy group (total 19, 12 women and 7 men) is taken into account considering the percentage of positive answers given to each cluster (Table 6) in the two testing periods before and after NIR stimulation and considering a mean of the answers given to each cluster in the period before NIR stimulation (Test 1) (Figure 7) and after NIR stimulation (Test2) (Figure 8).

Categories	% positive answers
Concentration	
Test 1	64%
Test 2	82%
Inattention/Distractibility	
Test 1	79%
Test 2	92%
Interpersonal intelligence	
Test 1	68%
Test 2	81%
Memory	
Test 1	72%
Test 2	87%
Memory for names	
Test 1	50%
Test 2	79%

Table 6: *Shows percentage of positive answers inside the 5 clusters by Cerebral Vasculopathy patients before (Test 1) and after (Test 2) NIR stimulation therapy.*

Table 6 show that in Test 2, after 1-month NIR stimulation, there is a considerable difference in perceived cognitive improvement in each cluster above all in the Memory cluster.

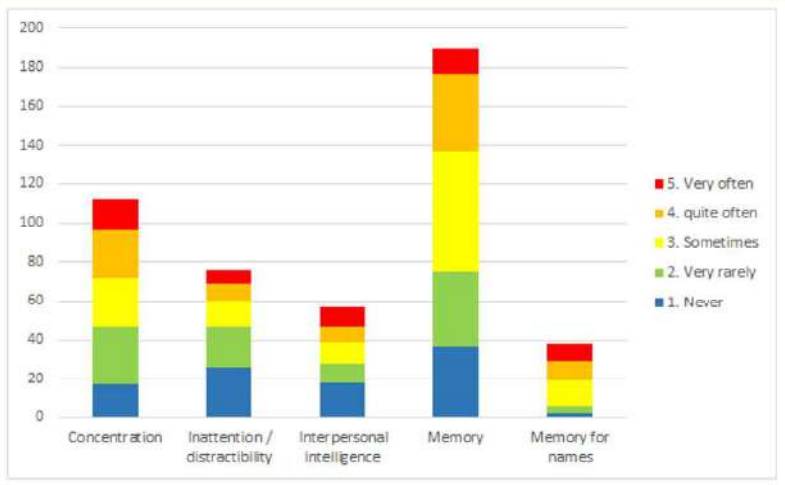

Figure 7: *Shows how many positive answers were given by the Cerebral Vasculopathy group to each question that were merged in the 5 cluster at Test 1 (before starting NIR treatment).*

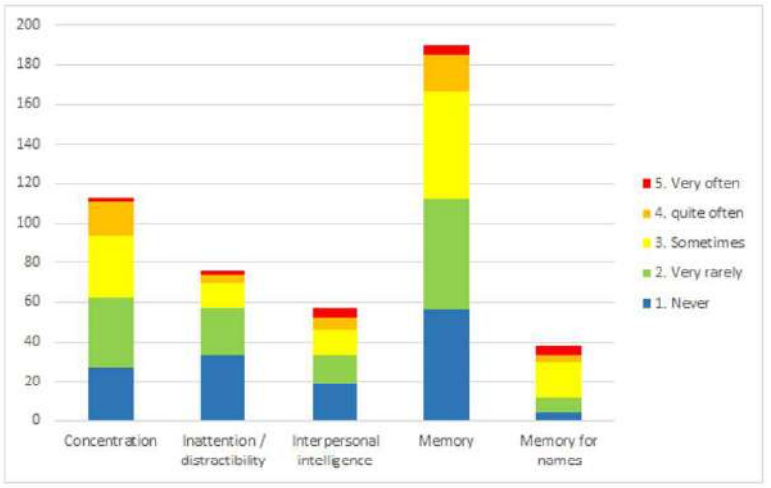

Figure 8: *Shows how many positive answers were given by the Cerebral Vasculopathy group to each question that were merged in the 5 cluster at Test 2 (after 1-month NIR treatment).*

Results according to pathology: Dementia

The Dementia group (total number 2 women) is taken into account since it is an important pathology to consider looking back at the scientific publications that are available. Data consists in the percentage of positive answers given to each cluster (Table 7) in the two testing periods before and after NIR stimulation and considering a mean of the answers given to each cluster in the period before NIR stimulation (Test 1) (Figure 9) and after NIR stimulation (Test2) (Figure 10).

Categories	% positive answers
Concentration	
Test 1	20%
Test 2	50%
Inattention/Distractibility	
Test 1	33%
Test 2	50%
Interpersonal intelligence	
Test 1	67%
Test 2	100%
Memory	
Test 1	26%
Test 2	53%
Memory for names	
Test 1	50%
Test 2	70%

Table 7: *Shows percentage of positive answers inside the 5 clusters by Dementia patients before (Test 1) and after (Test 2) NIR stimulation therapy.*

In this table 7, perceived improvements are highlighted in all the clusters. We are aware of the fact that this group is too small to be considered statistically significant, however important for only two persons to have a better perception of their quality of life after only 1-month NIR stimulation.

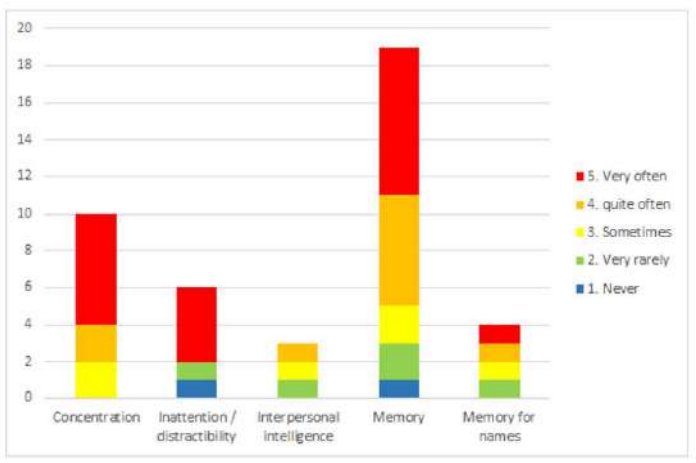

Figure 9: *Shows how many positive answers were given by the Dementia group to each question that were merged in the 5 cluster at Test 1 (before starting NIR treatment).*

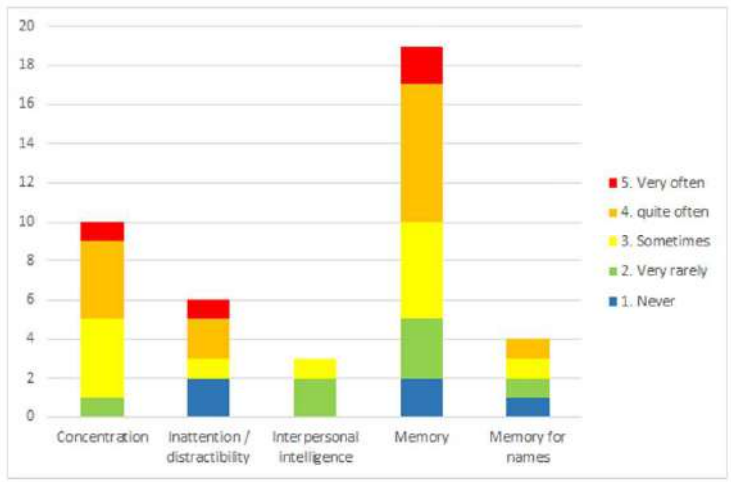

Figure 10: *Shows how many positive answers were given by the Dementia group to each question that were merged in the 5 cluster at Test 2 (after 1-month NIR treatment).*

Conclusion

This paper has the aim to describe a new device and its application in neurological disease. NIR device patent by Cerebro® is a helmet composed by 256 LEDs emitting 810 nm. This kind of wavelengths is described as activating different chromophores at the cortical level

promoting the modulation of cellular oxidative stress, neurometabolic and neuroinflammatory processes involved in different neurological disease. Our sample, since it is a pilot study, is small but may start to increase our work with this type of NIR device and proving the effect on everyday life in different diseases. The results from the compilation of the Cognitive Self-Assessment Questionnaire, in the two time period before (Test 1) and after (Test 2) NIR therapy, show a clear improvement in the perception of the symptoms in all the analyzed clusters: concentration, inattention/distraction, interpersonal intelligence and memory.

Specifically, Alzheimer's patients noticed a significant improvement in all the questions merged in the clusters with the greatest perceived improvement in the memory cluster. The same level of improved perception is observed by patients with Cerebral Vasculopathy. In patients with Dementia, the improvement is particularly evident in the "concentration" cluster. The other patients not mentioned in the result section for reductive sample size reason, also showed good improvements in all the clusters investigated.

From the data comparison between Test 1 and Test 2, pre and post NIR therapy, it is possible to hypothesize that the treatment with NIR, as described in Chapter 2, leads to a perception of improvement in cognitive abilities in patients suffering from neurological diseases.

Limitations of the Study

The limitations of this pilot study are certainly about the small sample size of the participants and the wide age range that was taken into account. It was not considered juxtaposing a control group since we considered a personal perception of cognitive symptoms and its eventual improvements after the NIR treatment.

This new Cerebro® patented technology, called NIR, allowed us to test this device and its efficacy on patients with neurological disorders by improving their quality of life and cognitive perception. The main bias of this study is relative to the submitted questionnaire based on the only individual perception of improvements. Future study on the use of NIR in neurological patients may add neuropsychological cognitive evaluations and anamnestic and structural data. The range of application of this device and this low light therapy in 810 nm is wide and this pilot study opens the way to new scenarios for NIR therapy and stimulation and possible application of this light therapy in support to neurological and neuropsychological rehabilitation.

Acknowledgements

We thank rehabilitative polyclinic Istituto San Celestino in Milan (Italy) and rehabilitative polyclinic "Giano" in Cesena (Italy) for the data collection and the support in completing this paper.

Conflict of Interest

M.D Samorindo Peci declares that he is the administrator of Cerebro® Innovative Neuroscientific Start-up whose NIR helmet was used in this study.

Bibliography

1. Heiskanen Vladimir and Michael R Hamblin. "Photobiomodulation: Lasers vs. Light Emitting Diodes?" *Photochemical and Photobiological Sciences* (2018).

2. Mester Andrew and Adam Mester. "The history of photobiomodulation: Endre Mester (1903-1984)". (2017): 393-394.

3. Sharma Sulbha K., *et al*. "Dose response effects of 810 nm laser light on mouse primary cortical neurons". *Lasers in Surgery and Medicine* 43.8 (2011): 851-859.

4. Ilic Sanja., *et al*. "Effects of power densities, continuous and pulse frequencies, and number of sessions of low-level laser therapy on intact rat brain". *Photomedicine and Laser Therapy* 24.4 (2006): 458-466.

5. Huang Ying-Ying., *et al*. "Biphasic dose response in low level light therapy". *Dose-response* 7.4 (2009).

6. Yip KK., *et al*. "The effect of low-energy laser irradiation on apoptotic factors following experimentally induced transient cerebral ischemia". *Neuroscience* 190 (2011): 301-306.

7. Abid Oueslati Blaise Lovisa, *et al*. "Photobiomodulation suppresses alpha-synuclein-induced toxicity in an AAV-based rat genetic model of Parkinson's disease". *PloS one* 10.10 (2015).

8. Ando Takahiro., *et al*. "Comparison of therapeutic effects between pulsed and continuous wave 810-nm wavelength laser irradiation for traumatic brain injury in mice". *PloS one* 6.10 (2011).

9. De Taboada Luis., *et al*. "Transcranial laser therapy attenuates amyloid-β peptide neuropathology in amyloid-β protein precursor transgenic mice". *Journal of Alzheimer's Disease* 23.3 (2011): 521-535.

10. Schiffer F., *et al*. "Psychological benefits 2 and 4 weeks after a single treatment with near infrared light to the forehead: a pilot study of 10 patients with major depression and anxiety". *Behavioral and Brain Functions* 5.1 (2009): 46.

11. Salehpour Farzad and Seyed Hossein Rasta. "The potential of transcranial photobiomodulation therapy for treatment of major depressive disorder". *Reviews in the Neurosciences* 28.4 (2017): 441-453.

12. Santana-Blank Luis., *et al*. "Quantum leap" in photobiomodulation therapy ushers in a new generation of light-based treatments for cancer and other complex diseases: perspective and mini-review". *Photomedicine and Laser Surgery* 34.3 (2016): 93-101.

13. Chen Yongmei, *et al*. "Thermal Effects of Transcranial near-Infrared Laser Irradiation on Rabbit Cortex". *Neuroscience Letters* 553 (2013): 99-103.

14. Purushothuman Sivaraman., *et al*. "Photobiomodulation with near Infrared Light Mitigates Alzheimer's Disease-Related Pathology in Cerebral Cortex - Evidence from Two Transgenic Mouse Models". *Alzheimers Research and Therapy* 6.1 (2014): 2.

15. Quirk Brendan J., *et al*. "Near-Infrared Photobiomodulation in an Animal Model of Traumatic Brain Injury: Improvements at the Behavioral and Biochemical Levels". *Photomedicine and Laser Surgery* 30.9 (2012): 523-529.

16. Liang Hl., *et al*. "Photobiomodulation Partially Rescues Visual Cortical Neurons from Cyanide-Induced Apoptosis". *Neuroscience* 139.2 (2006): 639-649.

17. Lee Hae In., *et al*. "Pre-Conditioning with Transcranial Low-Level Light Therapy Reduces Neuroinflammation and Protects Blood-Brain Barrier after Focal Cerebral Ischemia in Mice". *Restorative Neurology and Neuroscience* 34.2 (2016): 201-214.

18. Moreira Maria Stella., *et al*. "Effect of phototherapy with low intensity laser on local and systemic immunomodulation following focal brain damage in rat". *Journal of Photochemistry and Photobiology B: Biology* 97.3 (2009): 145-151.

19. Yan Xiaodong., *et al*. "Low-level laser irradiation modulates brain-derived neurotrophic factor mRNA transcription through calcium-dependent activation of the ERK/CREB pathway". *Lasers in Medical Science* 32.1 (2017): 169-180.

20. Xuan Weijun., *et al*. "Repeated transcranial low-level laser therapy for traumatic brain injury in mice: biphasic dose response and long-term treatment outcome". *Journal of Biophotonics* 9.11-12 (2016): 1263-1272.

21. Xu Zhiqiang., *et al*. "Low-level laser irradiation improves depression-like behaviors in mice". *Molecular Neurobiology* 54.6 (2017): 4551-4559.

22. Naeser Margaret A., *et al*. "Significant improvements in cognitive performance post-transcranial, red/near-infrared light-emitting diode treatments in chronic, mild traumatic brain injury: open-protocol study". *Journal of Neurotrauma* 31.11 (2014): 1008-1017.

23. Lapchak Paul A. "Taking a Light Approach to Treating Acute Ischemic Stroke Patients: Transcranial near-Infrared Laser Therapy Translational Science". *Annals of Medicine* 42.8 (2010): 576-586.

24. Henderson Theodore A and Larry Morries. "Near-Infrared Photonic Energy Penetration: Can Infrared Phototherapy Effectively Reach the Human Brain?" *Neuropsychiatric Disease and Treatment* (2015): 2191.

25. Freitas Lucas Freitas De and Michael R Hamblin. "Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy". *IEEE Journal of Selected Topics in Quantum Electronics* 22.3 (2016): 348-364.,

26. Hamblin Michael R and Tatiana N Demidova. "Mechanisms of Low Level Light Therapy". *Mechanisms for Low-Light Therapy* (2006).

27. Huang Ying-Ying., *et al*. "Low-level laser therapy (LLLT) reduces oxidative stress in primary cortical neurons *In vitro*". *Journal of Biophotonics* 6.10 (2013): 829-838.

28. Naeser Margaret A., *et al*. "Improved cognitive function after transcranial, light-emitting diode treatments in chronic, traumatic brain injury: two case reports". *Photomedicine and Laser Surgery* 29.5 (2011): 351-358.

29. Karu TI. "Molecular mechanism of the therapeutic effect of low-intensity laser radiation". *Lasers Life Sciences* 2.1 (1988): 53-74.

30. Cooper CE., *et al*. "Use of Mitochondrial Inhibitors to Demonstrate That Cytochrome Oxidase Near-Infrared Spectroscopy Can Measure Mitochondrial Dysfunction Noninvasively in the Brain". *British Journal of Pharmacology* 19 (1999): 27-38.

31. Hamblin Michael R. "Photobiomodulation for Alzheimer's Disease: Has the Light Dawned?". Photonics. Vol. 6. No. 3. Multidisciplinary Digital Publishing Institute (2019).

32. Hennessy Hamblin. "Photobiomodulation of the Brain: a New Paradigm (Conference Presentation)". Mechanisms of Photobiomodulation Therapy XII (2017).

33. Lane Nick. "Cell biology: power games" (2006): 901.

34. Waypa Gregory B., *et al*. "O2 sensing, mitochondria and ROS signaling: the fog is lifting". *Molecular Aspects of Medicine* 47 (2016): 76-89.

35. Zhao Yingzi., *et al*. "Vascular nitric oxide: Beyond eNOS". *Journal of Pharmacological Sciences* 129.2 (2015): 83-94.

36. Filiano Anthony J., *et al*. "Interactions of innate and adaptive immunity in brain development and function". *Brain Research* 1617 (2015): 18-27.

37. Hamblin Michael R. "Shining light on the head: photobiomodulation for brain disorders". *BBA Clinical* 6 (2016): 113-124.

38. Saltmarche Anita E., *et al*. "Significant improvement in cognition in mild to moderately severe dementia cases treated with transcranial plus intranasal photobiomodulation: case series report". *Photomedicine and Laser Surgery* 35.8 (2017): 432-441.

39. Berman Marvin H., *et al*. "Photobiomodulation with near infrared light helmet in a pilot, placebo controlled clinical trial in dementia patients testing memory and cognition". *Journal of neurology and Neuroscience* 8.1 (2017).

40. Oron Amir., *et al*. "Low-level laser therapy applied transcranially to rats after induction of stroke significantly reduces long-term neurological deficits". *Stroke* 37.10 (2006): 2620-2624.

41. Yun Yeong-Chan., *et al*. "Laser acupuncture exerts neuroprotective effects via regulation of Creb, Bdnf, Bcl-2, and Bax gene expressions in the hippocampus". *Evidence-Based Complementary and Alternative Medicine* 2017 (2017).

42. Moro Cécile., *et al*. "Photobiomodulation inside the brain: a novel method of applying near-infrared light intracranially and its impact on dopaminergic cell survival in MPTP-treated mice". *Journal of Neurosurgery* 120.3 (2014): 670-683.

43. Maloney Ryan., *et al*. "The application of low-level laser therapy for the symptomatic care of late stage Parkinson's Disease: a non-controlled, non-randomized study: # 185". *Lasers in Surgery and Medicine* 42 (2010).

44. Naeser M., *et al*. "Improved language after scalp application of red/near-infrared light-emitting diodes: pilot study supporting a new, noninvasive treatment for chronic aphasia". *Procedia-Social and Behavioral Sciences* 61 (2012): 138-139.

45. Ab Boonswang N., *et al*. "A new treatment protocol using photobiomodulation and muscle/bone/joint recovery techniques having a dramatic effect on a stroke patient's recovery: a new weapon for clinicians". *Case Reports* (2012): bcr0820114689.

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