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Red/near-infrared light-emitting diode therapy for traumatic brain injury

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ABSTRACT

This invited paper reviews our research with scalp application of red/near-infrared (NIR) light-emitting diodes (LED) to improve cognition in chronic, traumatic brain injury [1]. Application of red/NIR light improves mitochondrial function (especially hypoxic/compromised cells) promoting increased ATP, important for cellular metabolism. Nitric oxide is released locally, increasing regional cerebral blood flow. Eleven chronic, mTBI participants with closed-head injury and cognitive dysfunction received 18 outpatient treatments (MWF, 6 Wks) starting at 10 Mo. to 8 Yr. post-mTBI (MVA, sports-related, IED blast injury). LED therapy is non-invasive, painless, non-thermal (FDA-cleared, non-significant risk device). Each LED cluster head (2.1" diameter, 500mW, 22.2mW/cm^2) was applied 10 min (13J/cm^2) to 11 scalp placements: midline, from front-to-back hairline; and bilaterally on dorsolateral prefrontal cortex, temporal, and parietal areas. Testing performed pre- and post-LED (+1 Wk, 1 and 2 Mo post- 18th treatment) showed significant linear trend for LED effect over time, on improved executive function and verbal memory. Fewer PTSD symptoms were reported. New studies at VA Boston include TBI patients treated with transcranial LED (26J/cm^2); or treated with *only* intranasal red, 633nm and NIR, 810nm diodes placed into the nostrils (25 min, 6.5mW, 11.4J/cm^2). Intranasal LEDs are hypothesized to deliver photons to hippocampus. Results are similar to Naeser et al. (2014). Actigraphy sleep data show increased sleep time (average, +1 Hr/night) post- 18th transcranial or intranasal LED treatment. LED treatments may be self-administered at home (Naeser et al., 2011). A sham-controlled study with Gulf War Illness Veterans is underway.

Keywords: Traumatic brain injury, TBI, photobiomodulation therapy, TBI treatment, cognitive dysfunction, light emitting diodes LED, post-concussion syndrome, sports head injury, PTSD, LLLT

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1. INTRODUCTION

1.1 Mechanisms of low-level laser (light) therapy

There are several mechanisms associated with promoting physiological change following low-level laser, or LED (light) therapy (LLLT). [Note, in 2016, the MeSH term, photobiomodulation therapy, will be added for LLLT and defined as: "Treatment using irradiation with light of low power intensity so that the effects are a response to the light and not due to heat. A variety of light sources, especially low-power lasers are used."] The wavelengths commonly used with LLLT are red (600-700nm) and near-infrared (NIR, 800-900nm). When hypoxic/compromised cells are irradiated with red or NIR LLLT there is increased mitochondrial ATP production [3, 4]. Another change is release of nitric oxide from the hypoxic/compromised cells. In hypoxic cells, cytochrome-C oxidase (CCO), a membrane-bound protein that serves as the ultimate electron acceptor in the cell respiration, electron transport chain (among other functions), becomes inhibited by non-covalent binding of nitric oxide. When exposed to red or NIR photons, the CCO releases nitric oxide, which then diffuses outside the cell – increasing local blood flow and vasodilation [5, 6]. When traumatic brain injury (TBI) occurs, mitochondria become damaged within the cell [7], and there is mitochondrial dysfunction [8-10]. Increased focal, regional cerebral blood flow has been shown following application of NIR LLLT to the forehead in a severe TBI patient [11] and in chronic, major depression cases, including those with PTSD [12]. Some improvements were reported in both studies.

Also, following initial exposure to the red/NIR photons, there is a brief burst of reactive oxygen species (ROS) in the cell, and this activates a number of signaling pathways. The ROS leads to activation of redox-sensitive genes, and related transcription factors including NF-κβ [13, 14]. The LLLT stimulates gene expression for cellular proliferation, migration, and the production of anti-inflammatory cytokines and growth factors [15]. Vascular endothelial growth factor (VEGF), has been associated with enhanced post-ischemic neurogenesis (the formation of new neurons, or brain cells) in small animal studies, consistent with a possible role in ischemic brain tissue repair [16]. NIR laser therapy has been observed to increase VEGF and angiogenesis following myocardial infarction in animal studies [17]. Scalp application of red/NIR LLLT could stimulate secretion of VEGF, thus promoting angiogenesis and neurogenesis. VEGF could provide a therapeutic target for more chronic brain repair. Other studies have suggested that the anti-inflammatory, anti-edema and pro-angiogenic property of LLLT can also act as an effective treatment modality in stroke [15, 18].

Results from transcranial LLLT (tLLLT) studies treating acute, severe TBI in mice have suggested that increased *neurogenesis* (the formation of new neurons, or brain cells) and *synaptogenesis* (the formation of new synapses or connections between neurons) are among the most important mechanisms associated with improved recovery in TBI or stroke. For example, there was significantly better recovery at 28 days post- TBI in the mice receiving 3 daily NIR tLLLT treatments (beginning at 4 hours post- TBI), compared to controls [19, 20]. The increases in *neurogenesis* were located in the dentate gyrus of the hippocampus, and in the subventricular zone at day 7. Evidence of *synaptogenesis* was observed in the perilesional area and in the subventricular zone at day 28. Thus, results from these studies suggest that tLLLT can be used to support rehabilitation in patients suffering from the sequelae of TBI, as well as stroke. The potential for neurogenesis-targeted clinical therapy in TBI is a high priority [21].

2. TRAUMATIC BRAIN INJURY

2.1 Introduction to TBI

Traumatic brain injury (TBI) is a major medical problem worldwide, and in the U.S. three TBIs occur every minute [22]. Approximately 1.7 million patients are evaluated annually; over 5 million Americans are living with TBI-related disabilities. The annual cost is between $60-$76.5 billion [22, 23]. Closed-head, mild TBI (mTBI) is the most common (75%), and persistent cognitive dysfunction occurs in 5-22% of these cases. Mild TBI is associated with loss of consciousness (LOC) 30 min or less (including no LOC), and with a period of altered mental status, that could include post-traumatic amnesia - "memory gaps" or confusion lasting up to 24 hours.

2.2 Sports-related TBI

Cognitive dysfunction associated with sports-related mTBI is of increasing concern, both for males and females (including children) [24]. Within the past 10 years, a diagnosis of concussion in high school sports has increased annually,

by 16.5% [25]. With each successive concussion, there is a cumulative effect [26, 27] including a prolonged period of recovery, and a progressively increased risk for re-injury [28, 29]. Post- season verbal learning scores were observed to be lower than expected in 24% of college athletes who had participated in contact sports (vs. only 3.6%, in non-contact sports); and the greater the number of head impacts sustained, the slower the reaction time on ImPACT testing [27]. Among collegiate football players, those playing in the offensive linemen positions are at the greatest risk [30].

2.3 TBI in Soldiers and Veterans

Closed-head, blast injury is the signature injury of soldiers returning from Iraq and Afghanistan as part of Operation Enduring Freedom/Operation Iraqi Freedom (OEF/OIF) [31, 32]. The cognitive sequelae, recovery and rehabilitation are of increasing concern [33]. Estimates are as high as 320,000, regarding those who have returned with TBI [34, 35]. Post-traumatic stress disorder (PTSD) is also a major concern with OEF/OIF soldiers who have experienced mTBI [36]. One estimate is that 28% of those diagnosed with mTBI also report clinical levels of PTSD symptoms. A dose-response gradient for exposure to blast/combination mTBI on clinical levels of PTSD symptoms has been observed [37]. The incidence of mTBI with co-morbidities of PTSD and depression is higher in the military, than in civilians [38].

2.4 Diffuse Axonal Injury and White Matter Abnormalities on MRI

In most cases with closed-head mTBI, focal lesions are not present on CT scan, nor on structural MRI [26, 39-41]. However, 30% of cases without abnormality on CT scan have been observed to have abnormalities in white matter using Diffusion Tensor Imaging (DTI) MRI scans [42].

Diffuse axonal injury (DAI) or traumatic axonal injury (TAI), has been recognized as one of the main consequences of closed-head mTBI [43]. DAI results when shearing, stretching, and/or angular forces pull on axons and small vessels [10, 44-46]. The frontal lobes, including medial and lateral prefrontal cortex areas, are especially vulnerable to damage following mTBI where both linear and angular, acceleration/deceleration effects occur – e.g., whiplash in a motor vehicle accident (MVA), concussive blast force, or even direct impacts to the head [47-49]. This leads to impaired axonal transport, focal axonal swelling and (after several hours) may result in axonal disconnection [50]. All severities of TBI can result in a degree of axonal damage [51]. Functionally, the reduction or loss of interconnectivity produces the cognitive, emotional and behavioral problems observed following TBI [52, 53].

2.5 Development of Neurodegenerative Disease post- TBI

White matter degeneration, widespread tau and amyloid-beta pathology, and inflammation and may be present years after only a *single* TBI [54, 55]. Increased microglial activity may persist long-term post- TBI [56, 57]; persistent neuroinflammation may be a mechanistic link between TBI and the development of neurodegenerative disease, including Alzheimer's disease (AD) [55]. Damaged axons may serve as a source of the AD-associated protein amyloid-beta [46, 58, 59].

Chronic traumatic encephalopathy (CTE), a progressive tau protein-linked neurodegenerative disease, is believed to develop in part, from repeated head trauma [60, 61]. Symptoms include cognitive dysfunction, progressive irritability, suicidal ideation and dementia. It may develop years after the head trauma occurred. CTE has been documented in U.S. military veterans exposed to blast injury in Iraq and Afghanistan [49].

2.6 Functional Brain Imaging in TBI

Since 1999, mTBI cases have been observed on functional neuroimaging studies to have alterations in neural activation during performance of cognitive tasks [62]. While accuracy during a working memory (WM) task did not differ for the mTBI cases at 1 month post- mTBI, versus healthy adults, there was more widespread bifrontal and biparietal activation for the mTBI cases, even in the initial level of WM load. This pattern of abnormal hyperactivation was present even at 1 year post- mTBI [63, 64]. While the mTBI cases showed no significant post-concussive symptoms (PCS) at the one-year follow-up, they continued to show mildly slower reaction speed relative to healthy adults.

2.7 Resting state, functional connectivity MRI in TBI

Using a different type of fMRI scan – e.g. resting state, functional connectivity MRI (rs-fMRI) studies with healthy adults, have demonstrated specific *neural networks* that function in a wide-spread, but *temporally-coordinated manner* (with a very low frequency, 0.1 Hz), even while the subject is resting quietly in the MRI scanner without external, task-related stimuli [65, 66]. One of these networks, the Default Mode Network (DMN) consists in part, of the ventromedial prefrontal cortex (vmPFC); the precuneus and posterior cingulate cortex (precu/PCC); postero-lateral parietal cortices

(Angular gyri), and medial temporal lobes/hippocampi [67]. The DMN, including the precu/PCC in particular, shows rapid, and highly reactive *de-activation* in normals, during attentionally-demanding tasks. Abnormality in the DMN (with *failure to de-activate*) has been observed in mTBI cases [68-71].

Abnormalities have also been observed with mTBI cases in two other rs-fMRI networks, important for normal brain function during attention-related and cognitive tasks. The first is the salience network (SN) [72]. The SN controls the DMN, and the SN consists of the anterior insulae (AI), the pre-supplementary motor areas (preSMAs), and the dorsal anterior cingulate cortex (dACC) areas. The SN is critical for normal executive function and inhibition [73-75]. Brain cortex in the anterior parts of the SN (preSMAs and dACC) promote inhibitory control (*de-activation*) of the posterior parts of the DMN (precun/PCC), particularly during tasks that require *inhibition* and *rapid switching* for success. The SN is important for signaling the need to change behavior [76]. The second, additional rs-fMRI network is the central executive network (CEN). This network comprises dorsolateral prefrontal cortex areas and the intraparietal sulci [77, 78]. The CEN is particularly important, as the name implies, for executive function, including cognitive manipulation of temporal information, WM, processing speed, reasoning, problem solving, planning and execution, task flexibility and multi-tasking.

2.8 Cognitive Dysfunction in TBI

The most common complaints of cases with mTBI are in the domains of attention/concentration and WM - i.e., the ability to hold information in mind, and to manipulate it in light of incoming material [64, 79, 80]. At 6 months post-injury, indices of executive function have been found to predict persistence of post-concussive syndrome, in mild and moderate TBI patients [81]. One of the most debilitating sequelae of mTBI is the failed attempt to re-establish family and work relationships [82]. Due to the diffuse nature of damage, however, no single behavioral outcome measure captures the multidimensional nature of TBI outcome [83].

2.9 Sleep Disturbance in TBI

In addition to the cognitive and psychosocial issues post- TBI, there are problems with sleep [84-86]. An estimated 53% of individuals with TBI report sleep disturbances [87]. Sleep problems may exacerbate symptoms of TBI [88], increase neuropsychiatric symptoms (depression, anxiety) post-TBI [89], and interfere with participation in rehabilitation treatment [90]. Although the etiology of TBI-related sleep problems is currently under investigation, research has indicated neurobiological factors, specifically, impairment in the function of neural circuits involved in sleep/wake regulation [91, 92]. Among many factors, poor sleep would disrupt the normal, and necessary clearing of metabolites including beta-amyloid and other potentially neurotoxic waste products that accumulate during the awake central nervous system [93]. The brain lacks a lymphatic vasculature to carry out this function.

2.10 Pharmacologic Treatments for TBI

Pharmacologic treatments for TBI have been mostly unsuccessful. [94] There are some pharmacologic interventions available for systemic and intracranial changes associated with moderate and severe TBI, with few controlled systematized studies for pharmacologic treatment of cognitive impairment. [95] There was a large clinical trial (COBRIT) for moderate, severe and complicated mTBI, which utilized the pharmacologic agent, Citicoline. [83] Citicoline was evaluated as a neuroprotective agent and in TBI has previously demonstrated some efficacy in secondary measures for stroke, and smaller TBI studies. [83] In 2012, the results of the COBRIT study showed the use of citicoline compared with placebo for 90 days did not result in improvement in functional and cognitive status.[96]

There are currently no pharmacologic treatments for mTBI secondary injury or for prevention of cognitive and behavioral problems associated with mTBI. [97, 98] Further investigation is warranted to examine the effects of cholinesterase inhibitors, with preliminary evidence suggesting improvement in attentional difficulties and mixed results for memory treatment. [82] Regarding executive dysfunction, no conclusions can be drawn for improvement by pharmacologic intervention post- TBI. [82, 95]

McAllister et al (2011) examined the effect of Bromocriptine, a dopamine D2 receptor agonist on WM performance in healthy controls versus mTBI patients. Bromocriptine was associated with improved WM performance only in healthy controls, not mTBI patients. Imaging showed that mTBI patients were not able to recruit WM task-specific regions of interest. These results suggest that mTBI patients may have altered response to dopamine. In another study, McAllister et al (2011)found the opposite effect, using Guanfacine, an alpha-2 adrenergic receptor agonist. Guanfacine was found to

selectively improve WM performance in mTBI but not in healthy controls. In the mTBI group, increased activation was observed within a WM task-specific region of interest. This pharmacologic agent may be a promising pharmacologic agent to test hypotheses about the neural mechanisms of cognitive dysfunction after mTBI.

2.11 Cognitive Rehabilitation Therapies for TBI

Review of the effectiveness of current cognitive and behavioral treatments to improve executive function after TBI have shown limited evidence for the efficacy of cognitive rehabilitation [33, 101-103]. Executive dysfunction continues to present a challenge to the rehabilitation process [104]. Behavioral treatment approaches for TBI patients attempt to maximize the patient's behavioral functioning by working with residual brain-based capacities, but injured, non-functioning brain cells may limit their potential for success. Treatments are needed that *directly target injured brain cells* to improve the functioning of underlying brain systems (including functional connectivity in networks such as the DMN, SN, and CEN) that regulate attention, executive function, memory, emotions and behavior. Effective treatments to improve cognition in individuals with TBI are currently lacking, and these are urgently needed for veterans, as well as non-veterans. Transcranial red/NIR LLLT is a promising clinical research method to fill these needs.

3. METHODS

3.1 Transcranial LED Treatment to Improve Cognition in chronic, mTBI – Case Reports

Naeser et al., (2011) described two case reports for chronic, mTBI where cognition improved following treatment with red/NIR light-emitting diodes (LEDs). The LEDs were applied transcranially to forehead and scalp areas including midline, mid-sagittal areas; as well as bilateral frontal, temporal, parietal and occipital areas. Each red/NIR LED cluster head (MedX Health, Toronto) had a 5.35 cm diameter, and was 500 mW containing 61 diodes (9x633 nm, and 52x870 nm). The power density was 22.2 mW/cm^2, and 13 J/cm^2 (CW) was applied to the scalp from each LED cluster head for approximately 10 min (estimated 0.4 J/cm^2 to surface brain cortex).

Patient 1 (59 Yr.F, professor of web design) began transcranial LED (tLED) treatments seven years after a closed-head mTBI from a motor vehicle accident (MVA). Pre-tLED, her ability to sustain attention (computer work) lasted only 20 min. After eight weekly tLED treatments, her sustained attention on the computer increased to 3 hours. She reported that if she stopped treating for more than 2 Wks, she regressed.

Patient 2 (52 Yr.F, high-ranking, retired military officer) had a history of closed-head mTBIs (sports/military, and recent fall onto concrete from a swing). Her structural MRI brain scan showed moderate fronto-parietal atrophy, for her age. Pre-tLED, she was on medical disability for 5 months. After 4 months of nightly tLED treatments at home, her medical disability was discontinued; she returned to work full-time as an executive consultant with an international technology-consulting firm. Neuropsychological testing after 9 months of tLED indicated significant improvement (+1SD) in executive function (inhibition, inhibition accuracy) and (+2SD) in memory, as well as reduction in post-traumatic stress disorder (PTSD). Patient 2 reported that if she stopped treating for more than 1 Wk, she regressed. Both patients continued home treatments with tLED for at least 5 years; there have been no adverse events or negative side effects. P1 is lost to follow-up, and P2 continues with home treatments.

3.2 Transcranial LED Treatment to Improve Cognition in chronic, mTBI – Open Protocol Group Study

Naeser et al., (2014) conducted a pilot, open-protocol study with a larger number of chronic, mTBI patients, to examine whether tLED with the same red/NIR LED cluster heads could improve cognition when a systematic treatment protocol was used. Eleven chronic, mTBI participants (26–62 years of age, 6M) with non-penetrating brain injury and persistent cognitive dysfunction were treated for 18 outpatient sessions (Monday, Wednesday, Friday, for 6 Wks), starting at 10 months to 8 years post-mTBI (MVA or sports-related; and one participant, improvised explosive device blast injury). Four had a history of multiple concussions. Each LED cluster head (5.35 cm diameter, 500 mW, 22.2 mW/cm^2) was applied for 9 min 45 sec to each of 11 scalp placements (13 J/cm^2, CW). LEDs were placed on the midline from front-to-back hairline; and bilaterally on frontal, parietal, and temporal areas. Six LED cluster heads were applied simultaneously, held in place with a soft nylon cap. Each participant was treated in a recliner chair. See Naeser et al., (2014) for specific LED placements. These placements covered nodes located on the DMN, SN, and CEN. It was hypothesized that these placements would increase ATP and improve focal, regional cerebral blood flow in these cortical areas. Neuropsychological testing was performed pre-LED, and at 1 week, and 1 and 2 months after the 18th tLED treatment.

3.2.1 Results

A significant linear trend was observed for the effect of tLED treatment over time for the Stroop (Color Word Interference) test for Executive Function, Trial 3 inhibition (p = 0.004); Stroop, Trial 4 inhibition switching (p = 0.003); California Verbal Learning Test (CVLT) II, Alternating Versions, Total Trials 1–5 (p = 0.003); and CVLT-II, Long Delay (20 min) Free Recall (p=0.006). See Fig. 1. Participants reported improved sleep, and fewer post-traumatic stress disorder (PTSD) symptoms, if present (Fig. 2). Participants and family reported better ability to perform social, interpersonal, and occupational functions.

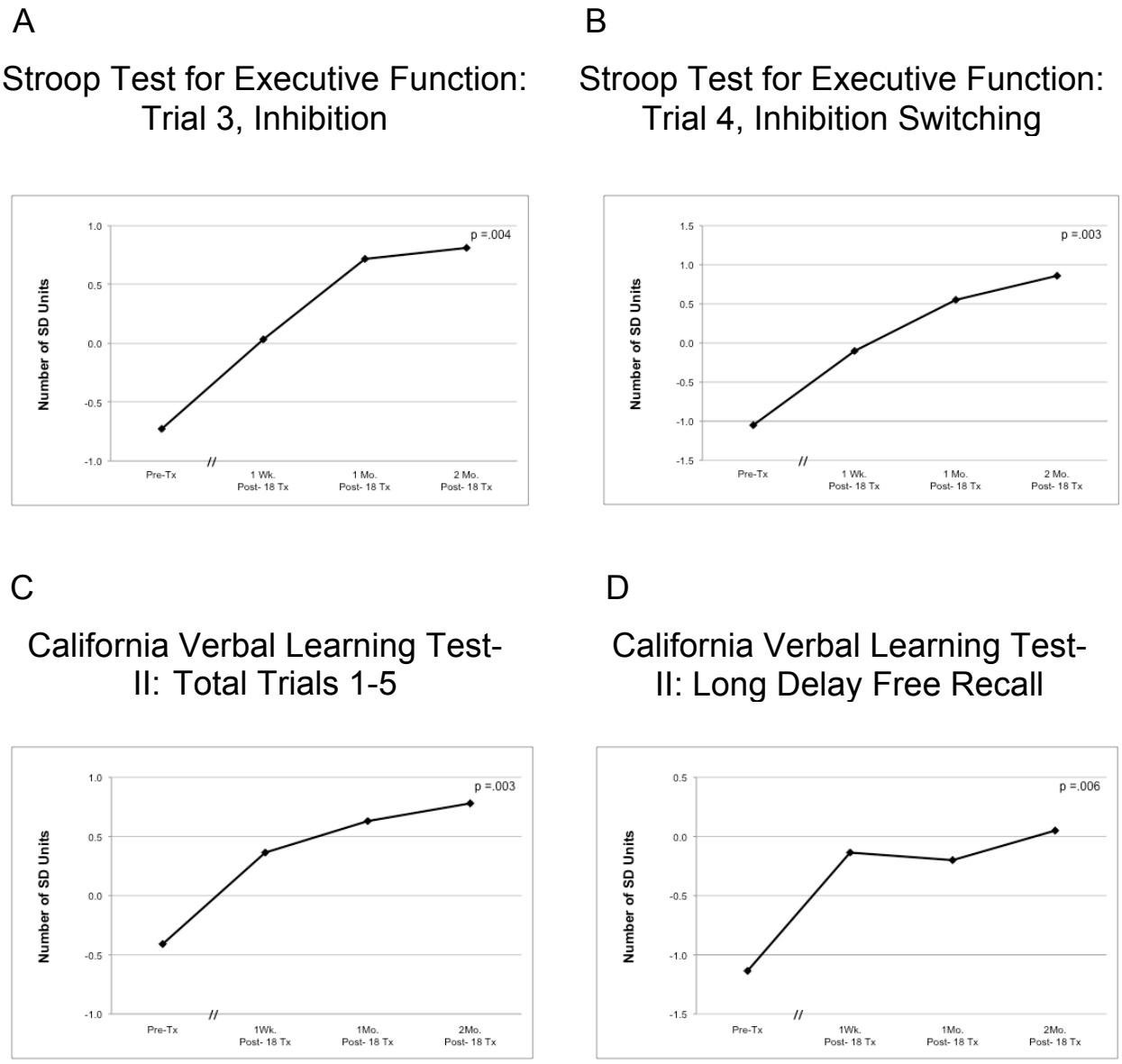

Fig. 1. Graphs showing a significant linear trend over time, for the effect of transcranial LED treatments on specific neuropsychological tests. A) Stroop (color-word interference test) for Executive Function: Trial 3, inhibition (p=.004); B) Stroop, Trial 4 inhibition switching (p=.003); C) California Verbal Learning Test (CVLT-II), Alternating Versions, Total Trials 1-5 (p=.003); and D) CVLT-II, Long Delay (20 min) Free Recall (p=.006).

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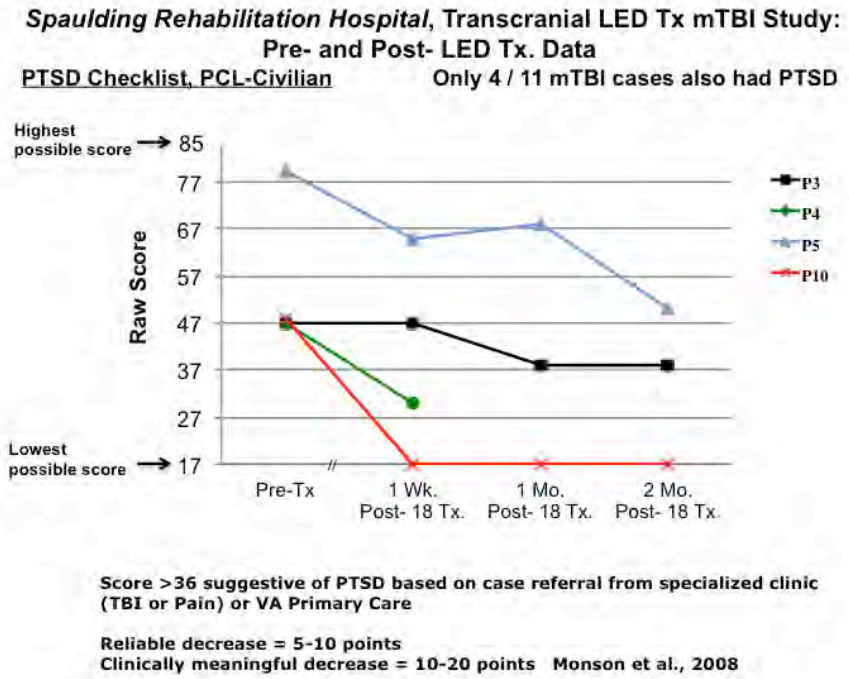


Fig. 2. Four of the eleven mTBI cases treated in the Naeser et al., 2014 study, also had post-traumatic stress disorder (PTSD). All four cases showed a clinically meaningful or reliable decrease in symptoms of PTSD, after the transcranial, red/near-infrared LED treatment series.

Source: Data are graphed from: Naeser, Zafonte, Krengel, Martin, Frazier, Hamblin, Knight, Meehan, Baker, J. Neurotrauma, 2014.

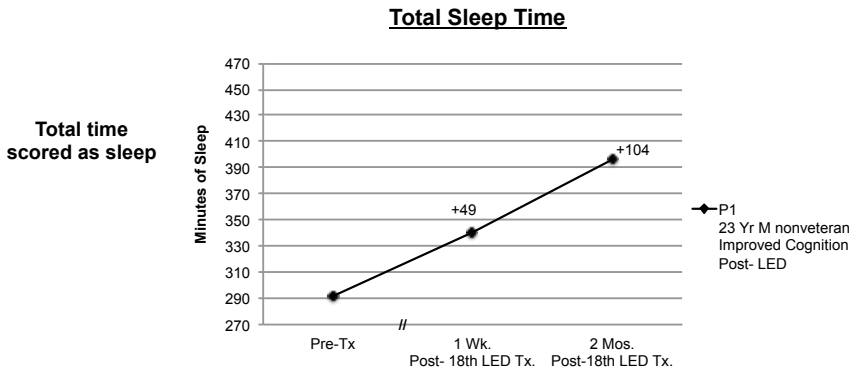
3.3 Transcranial LED Treatment to Improve Cognition and Sleep - Pilot Study, VA Boston Healthcare System

Bogdonova, et al., (2014) studied the effects of tLED treatment on sleep and cognitive function in patients with chronic moderate TBI, at the VA Boston Healthcare System. Two patients (1 F) with moderate TBI and persistent cognitive dysfunction (at least 2 SD below average on one, or 1 SD below average on at least two neuropsychological tests of executive function and memory) received 18 sessions of tLED therapy (M,W,F for 6 Wks, with at least 48 hours between sessions). Both cases treated with tLED showed marked improvement in sleep by increasing an average of 1 hour per night (measured with Actigraphy), at 1 Wk post- the tLED treatment series, as compared to pre- tLED. P1 also improved in executive function, verbal memory, and sleep efficiency; while P2 improved on measures of PTSD (PCL-M), and depression. No adverse events were reported. See Figs. 3-6.

Boston VA Medical Center, LED Tx. TBI, blast TBI (and TBI+PTSD)

Actigraphy Sleep Data

P1, 23 Yr M (Nonveteran) ***Moderate TBI***
Enter Study at 13 Mo. *Post- Bicycle/Car accident*
Pre- and Post- 18 ***Transcranial*** LED Treatments



Bogdanova Y, Martin PI, Ho MD, Krengel MH, Ho V, Yee MK, Knight JA, Hamblin MR, Naeser MA. Poster, ACRM Meeting, Oct. 2014

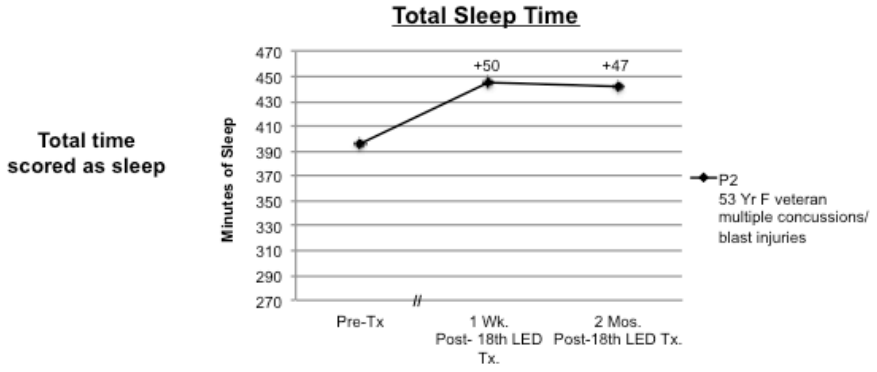
Fig. 3. Time spent asleep, increased by about 1 hour after 18 transcranial red/NIR LED treatments. The Actigraphy watch was worn 24/7 for 1 week pre-tLED, and again at 1 Wk and 2 months post-tLED. This individual with moderate TBI also improved on executive function and verbal memory.

Source: Bogdanova Y, Martin PI, Ho MD, Krengel MH, Ho V, Yee MK, Knight JA, Hamblin MR, Naeser MA. Arch Phys Med Rehabil 2014, p. e77.

Boston VA Medical Center, LED Tx. TBI, blast TBI (and TBI+PTSD)

Actigraphy Sleep Data

P2, 53 Yr F (Veteran) mTBI+PTSD
Enter Study at 2.5 Yrs. Post- Multiple Concussions and ***IED Blast Injuries (30 - 50)***
Pre- and Post- 18 ***Transcranial*** LED Treatments



Bogdanova Y, Martin PI, Ho MD, Krengel MH, Ho V, Yee MK, Knight JA, Hamblin MR, Naeser MA. Poster, ACRM Meeting, Oct. 2014

Fig. 4. Time spent asleep, increased by about 1 hour after 18 transcranial red/NIR LED treatments. The Actigraphy watch was worn 24/7 for 1 week pre-tLED, and again at 1 Wk and 2 months post-tLED. This woman also had reduced symptoms of PTSD and depression post- tLED, see Fig.'s 5 and 6.

Source: Bogdanova Y, Martin PI, Ho MD, Krengel MH, Ho V, Yee MK, Knight JA, Hamblin MR, Naeser MA. Arch Phys Med Rehabil 2014, p. e77.

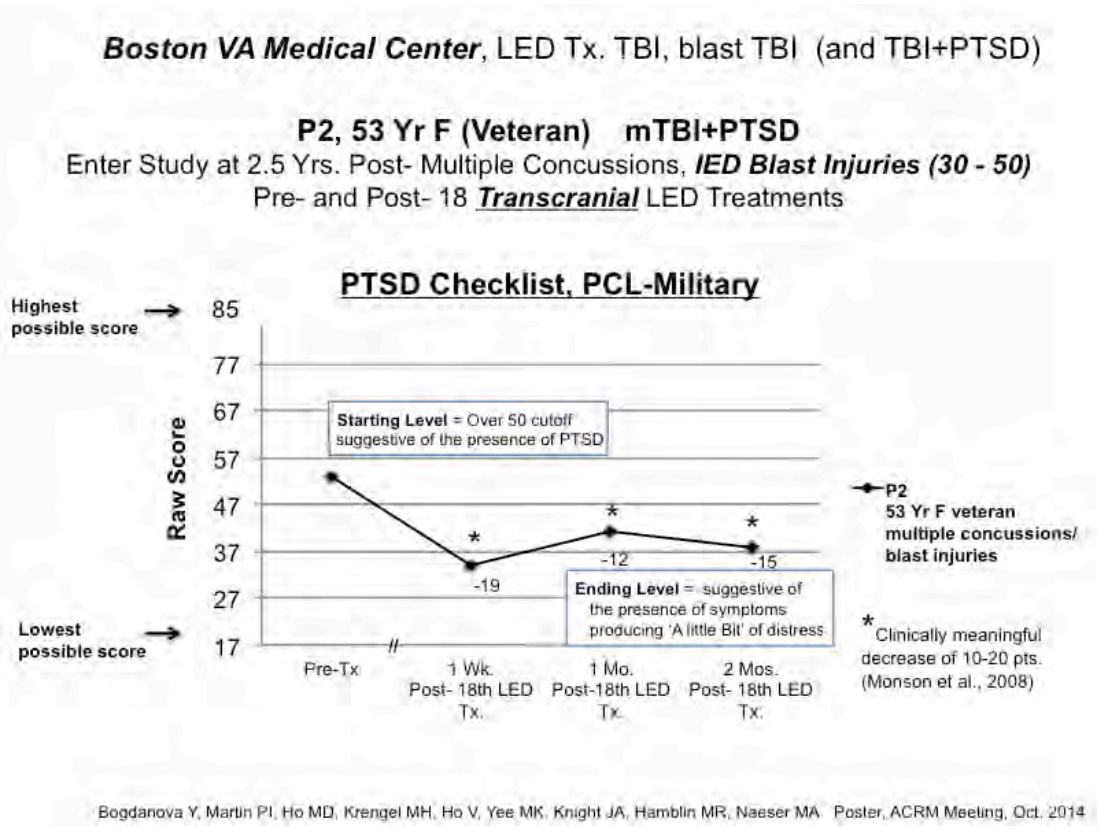


Fig. 5. Reduced PTSD symptoms after 18 transcranial red/NIR LED treatments. The changes showed a clicically meaningful decrease in PTSD symptoms at 1 week and 2 months post- tLED, compared to pre- tLED.

Source: Bogdanova Y, Martin PI, Ho MD, Krengel MH, Ho V, Yee MK, Knight JA, Hamblin MR, Naeser MA. Arch Phys Med Rehabil 2014, p. e77.

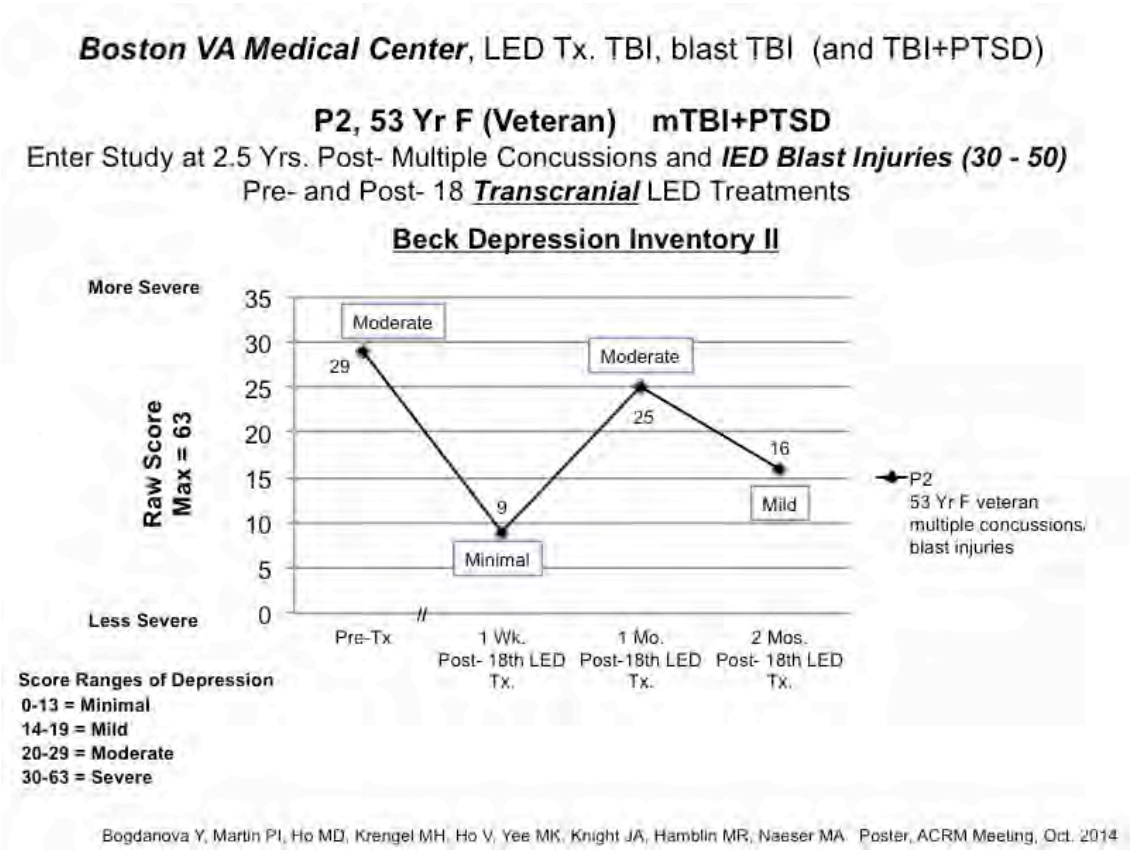


Fig 6. Reduced levels of depression after 18 transcranial red/NIR LED treatments in this woman veteran with mTBI+PTSD. Pre- tLED, the depression level was rated as moderate. The depression level was rated as minimal at 1 week post- the 18th treatment. Although the depression returned to moderate at 1 month post- the 18th tLED treatment, it was rated as only mild at 2 months after the last tLED treatment. Results might have been more consistent, if she had access to LED devices for home treatment [2].

Source: Bogdanova Y, Martin PI, Ho MD, Krengel MH, Ho V, Yee MK, Knight JA, Hamblin MR, Naeser MA. Arch Phys Med Rehabil 2014, p. e77.

3.4 *Intranasal* LED Treatment to Improve Cognition and Sleep - Pilot Study, VA Boston Healthcare System

A pilot, open-protocol, *intranasal* LED research project with mTBI participants who have chronic, cognitive dysfunction is in progress at the VA Boston Healthcare System (Naeser lab, personal observation). Two, small diodes (one clipped into each nostril) are used simultaneously for 25 min. The red, 633nm, intranasal diode is 6.5 mW (CW), 7.6 mW/cm^2, with estimated energy delivery to mucosa, 11.4 J/cm^2 (Vielight, Toronto). See Fig. 7A. The NIR, 810nm, intranasal diode has the same parameters, except it is pulsed at 10Hz (6.5 mW, net of 50% pulse duty cycle). Both Intranasal LEDs are noninvasive, painless and nonthermal. They each use one AA battery (1.4V) for power.

It is hypothesized that some NIR photons can reach the hippocampal/lateral entorhinal cortex areas via intranasal delivery. The red photons are hypothesized to improve blood rheology [106] and to improve sleep by increasing melatonin [107]. Participants are treated 3x/Wk for 6 Wks; with 48 hours between treatments. The pre- and post- intranasal LED testing is the same as for mTBI cases who receive tLED treatments [1, 105].

Thus far, one mTBI participant (24 Yr.F) with a history of four sports-related concussions (2 snow boarding and 2 field hockey) has received the intranasal LED treatment series. The results post- the intranasal series were similar to those for our tLED studies [1, 2, 105]. Significant improvements were observed on tasks of executive function (Fig. 7B), verbal memory (Fig. 7C,D), attention and verbal fluency, at 1, 6 and 12 Wks post- the 18th intranasal LED treatment. At one-

Wk post- the 18th intranasal LED treatment, the participant's average total sleep time had increased by 61 min per night (Fig. 8), and her sleep efficiency (total sleep time/total time in bed) had increased by 11%. Her sleep efficiency at 12 Wks post- Intranasal LED was 5% above pre- intranasal LED levels, and she reported no longer using any sleep medication that she had previously been using regularly. It is possible that her sleep parameters would have continued to improve even more, if she had the opportunity for self-administered home treatment with the intranasal LEDs. There were no negative side effects or complications.

Boston VA Medical Center, LED Tx. TBI, blast TBI (and TBI+PTSD)
P3, 24 Yr F (Nonveteran) ***Mild TBI***
3 Yrs. Post- TBI *4 Sports-related Concussions (snow boarding; field hockey)*
Pre- and Post- 18 ***Intranasal Only*** *(Red and NIR)* LED Treatments M,W,F 6 Wks.

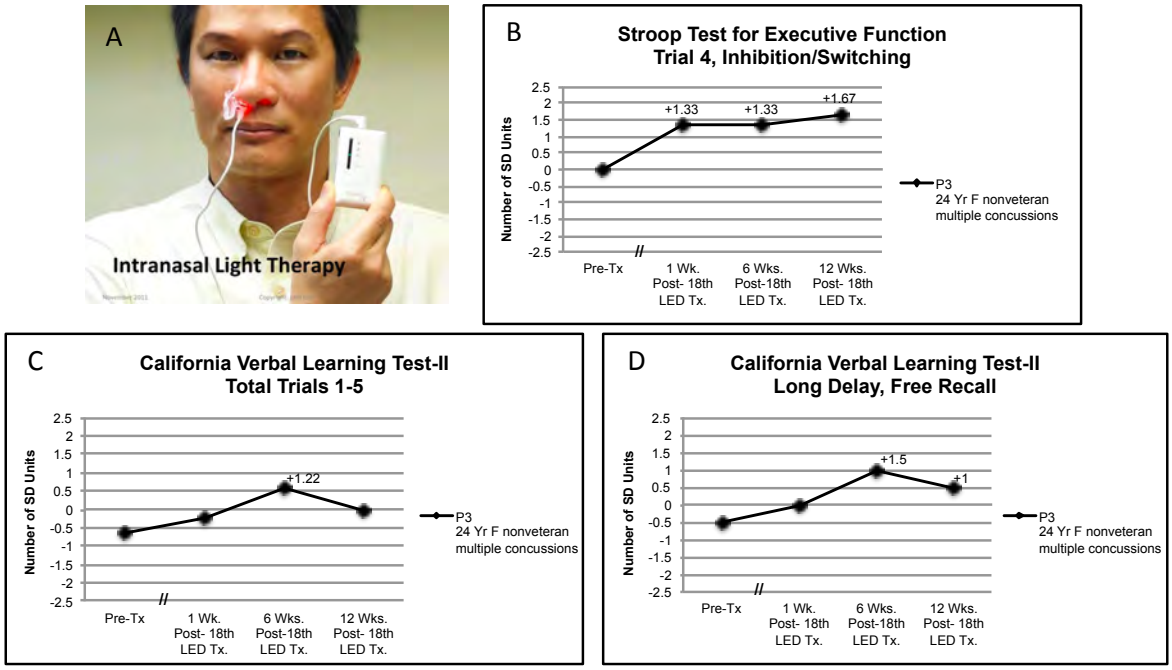


Fig. 7. Improvements in executive function and verbal memory after the 18th intranasal LED treatment in this 24 Yr.F who had a history of 4 mild TBIs. Improvements were +1 SD or more, on these tests at 1 Wk, 6 and/or 12 Wks post the 18th intranasal LED treatment.

Source: Bogdanova Y, Martin PI, Ho MD, Yee MK, Ho VT, Knight JA, Hamblin MR, Naeser MA. Poster for North American Brain Injury Society Meeting, San Antonio, April 2015.

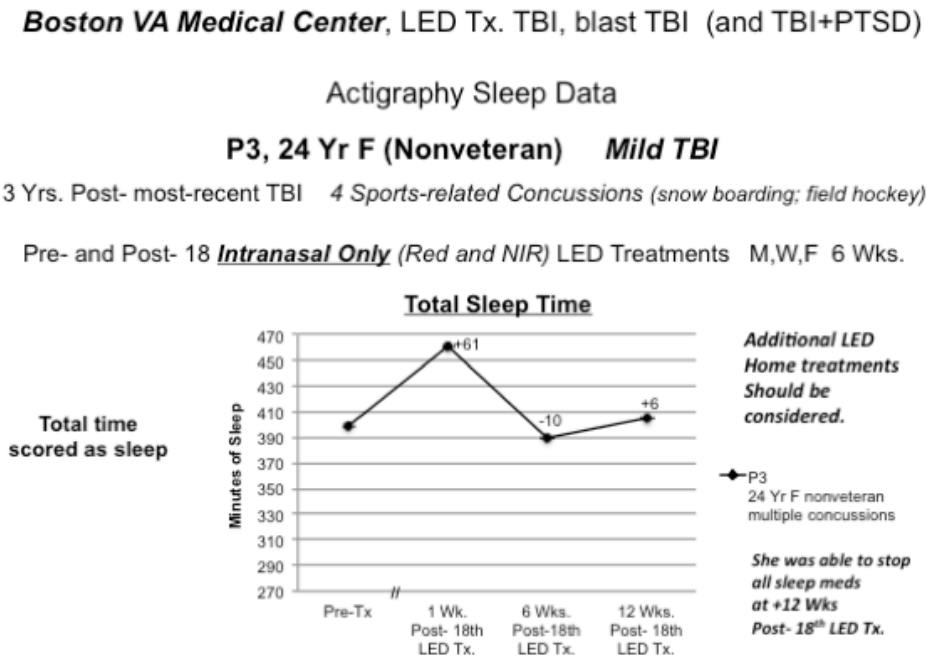


Fig. 8. Time spent asleep, increased by about 1 hour after 18 intranasal red/NIR LED treatments. The Actigraphy watch was worn 24/7 for 1 week pre- intranasal LED, and again at 1 week, 6 and 12 weeks after the 18th intranasal LED treatment. The gains made at 1 week post- the intranasal treatments (but not sustained at 6 and 12 weeks post- the last intranasal treatment) might have been sustained if she had had access to the intranasal LEDs for home treatments.

Source: Bogdanova Y, Martin PI, Ho MD, Yee MK, Ho VT, Knight JA, Hamblin MR, Naeser MA. Poster for North American Brain Injury Society Meeting, San Antonio, April 2015.

4. DISCUSSION

Major findings from the above-mentioned open-protocol, tLED studies to improve cognition in chronic mTBI cases were significant improvements in executive function, and in verbal learning and memory. In the Naeser et al. (2014) study, the cases had experienced persistent cognitive dysfunction, ranging from 10 months to 8 years. As is common with mTBI cases, heterogeneity was present among the 11 cases [83], including four cases with a history of multiple concussions. These findings are discussed separately, below and possible mechanisms associated with beneficial effects post- tLED are offered.

4.1 Executive Function, and Relationship to resting state, functional connectivity MRI networks (DMN and SN)

In the area of executive function (Trial 4, Inhibition Switching, the most difficult of the Stroop tasks), there was variability in the entry levels across the mTBI cases. For example, in 5/9 cases (56%), the Pre- LED levels were at least -1.0 SD below average; whereas 4/9 entered with average scores (age- and education-adjusted norms). All five cases who entered with below-average scores on the Stroop Trial 4, improved by +1 to +4.5 SD at 2 months post- tLED.

Variability in performance on Stroop, Inhibition Switching was observed among a large number of TBI cases that were studied with rs-fMRI, fMRI and DTI [72]. In that study, 20/46, 43% also performed poorly on the "stop-signal reaction task" (SSRT) with slower response inhibition (higher SSRT response times). These cases with slower reaction times in the NoGo condition were observed to have *failure in deactivating* the DMN, particularly the precu/PCC portion. Other parts within the DMN including vmPFC and left hippocampus were also observed to have less deactivation. Failure to properly deactivate the DMN during cognitive tasks that require rapid shifting of attention and inhibition has also been observed in several other studies with TBI cases [68-71].

The Bonnelle et al., (2012) study, however, also observed abnormalities in the SN in their TBI cases; particularly among those cases who *failed to deactivate the precu/PCC* during the SSRT task. The SN consists of the AI, preSMA and dACC; and the SN regulates activity in the DMN. For example, these authors observed that *failure to de-activate precun/PCC* during the SSRT task was predicted by the amount of white matter damage in the SN – i.e., significantly

lower fractional anisotropy (FA) values on DTI scans were observed in the SN tract connecting the right AI to the preSMA and dACC in these TBI cases. They observed a significant linear correlation between the right AI-preSMA/dACC tract FA, with the amount of precu/PCC activation during stopping ($r = -0.472$, $p < 0.0005$). Furthermore, and most relevant to results in the present study, they observed that on the Stroop [108] that "the FA within the rAI–preSMA/dACC tract (corrected for age and whole-brain white matter damage) was significantly correlated with the *inhibition/switching* vs. combined color naming and word reading contrast score (Spearman one tailed $r = -0.265$ $P = 0.029$ $n = 52$)." The SN was not correlated with any other neuropsychological measure.

Thus, at least for the five mTBI cases in the Naeser et al., 2014 study who entered with below-average Stroop, Inhibition Switching scores, but who also improved by at least +1 to +4.5 SD, post- tLED, it is possible that the red/NIR photons from the LED cluster heads that had been placed extracranially (particularly on midline placements) affected some nodes within the SN, and/or the DMN, thus improving function of these nodes, and/or connections between these nodes. Possible underlying mechanisms and physiological changes post- tLED, such as increase of ATP in hypoxic/compromised cells that are part of the DMN or SN; or local increase in rCBF, are discussed later. These are cellular changes that could have supported the behavioral improvement in executive function (Stroop Trial 4, Inhibition Switching) Post- LED.

Transcranial LED and fMRI research in our lab has recently observed focal, increased activation in targeted cortical areas on fMRI scans, post- 18 transcranial red/NIR LED treatments with chronic, left-hemisphere stroke patients [109]. Thus, our results with stroke patients suggest that the LED placement loci may have a focal effect, subjacent to the LED placement locations. Although no rs-fMRI, or task-related fMRI studies were part of our pilot studies with chronic mTBI cases, specific LED placements may have had a beneficial, focal effect on specific nodes within the SN and the DMN.

4.1.1 Specific tLED placements may affect specific parts of the SN and DMN in TBI Cases

Specific LED placements that may have treated specific nodes within the SN include the following: 1) The LED placement on the midline of face, centered over the forehead and front hairline, likely targeted the left and right dACC nodes within the SN. 2) The LED placement on the midline, vertex of the head likely also targeted the left and right preSMA nodes within the SN. 3) The LED placements on the left and right temple areas may have reached the AI within the SN. This is unknown, however, due to the greater depth of the anterior insula.

Specific LED placements that may have treated specific nodes within the DMN include the following: 1) The LED placement on the midline of face, centered over the forehead and front hairline likely targeted the left and right vmPFC nodes within the DMN. 2) The LED placement on the scalp midline, superior to the external occipital protuberance (and half-way towards the vertex), likely targeted the left and right precuneus areas, part of the precu/PCC node of the DMN. 3) The LED placements, posterior and superior to each ear, likely targeted the left and right postero-lateral, inferior parietal cortex (Angular gyrus areas), also nodes within the DMN.

Appropriate increased activation in the CEN explained below, would also be important for improved behavior on executive function, Stroop, Trial 4, Inhibition Switching. The increased activation in the CEN can only occur, however, when there is appropriate decreased activation in the precun/PCC.

4.2 Verbal Learning and Memory, and Relationship to resting state, functional connectivity MRI (CEN)

The CVLT is a verbal WM task where increased activation on task-related fMRI is associated with dorsolateral prefrontal cortex (DLPFC), and/or fronto-parietal areas [110, 111]. Working memory is associated with the CEN on rs-fMRI [73]. The CEN is a fronto-parietal system consisting primarily of the DLPFC and posterior parietal cortex (PPC) [77]. In addition to WM, the CEN is important for high-level cognitive functions such as planning, decision-making, and control of attention [78]. During WM tasks such as the CVLT, the CEN should be activated, however, the DMN should be deactivated, with a coordinated toggling back and forth between the two networks.

In the Naeser et al., 2014 study, all three cases who entered with scores at least -1 SD below average on the CVLT, Total Trials 1-5, improved by +1 to +2 SD at 2 months Post- LED. Also, a total of 5/7 cases who entered with scores at least -1 SD below average, on the CVLT, Long Delay Free Recall, improved by +1 to +3.5 SD at 2 months post- tLED.

4.2.1 Specific tLED placements may affect specific parts of the CEN in TBI Cases

Although no rs-fMRI, or task-specific fMRI studies were part of this pilot study, specific LED placements may have had a beneficial, focal effect on specific nodes within the CEN. These include the following: 1) The LED placements, located immediately posterior to the left and right front hairline, likely targeted the DLPFC areas; and 2) the LED placements, located posterior and superior to each ear, likely targeted the inferior parietal cortex/posterior parietal cortex (IPC/PPC, Angular gyrus) areas. Each of these fronto-parietal LED placement areas represent nodes within the CEN.

The two patients who had showed no change on the Stroop, Trial 4, Inhibition Switching at 2 months Post- LED (P7, P9), both showed improvement, however, on the CVLT tests - e.g., +1.5 on the Total Trials, 1-5; and +1 SD on the Long Delay Free Recall. Thus, every patient who entered the Naeser et al., (2014) study (regardless of severity level at entry) improved by at least +1 SD on either the Stroop, Trial 4, Inhibition Switching (most cases, 7/9); or on the CVLT tests (7 cases improved on the CVLT Long Delay Free Recall). P7 and P9 who had not improved on Stroop, Trial 4, Inhibition Switching improved by at least 1 SD on both CVLT tests – e.g., Total Trials 1-5, and on Long Delay Free Recall. P7 and P9 had suffered multiple concussions, and the disruption of white matter pathways and functional connectivity patterns for these two mTBI cases was likely quite variable.

4.3 Depression

There was only a trend for significant change in depression at the 1-Wk post- tLED testing ($p = .045$), and not an overall linear effect at 2 months post- tLED. A total of only 5 cases had entered the study with moderate or severe depression. The pattern of initial reduction in depression at 1-Wk post- tLED in 4/5 of these cases (but not an overall lasting change at 1 or 2 months post- tLED), is similar to the results observed in the Schiffer et al., (2009) study with 10 severe depression cases, where depression was significantly reduced at 2 Wks post- a single, NIR tLED treatment to the left and right forehead areas, but scores returned towards baseline at 4 Wks post-tLED. In both the Schiffer study and Naeser et al., (2014), however, most of the post- tLED depression scores did not return to the pre- LED levels. Some studies have suggested that there is downregulation of neurogenesis in the hippocampus in major depression [112]. The potential role of tLED (or intranasal LED) to upregulate neurogenesis in the hippocampus requires further study. Our data, as well as that from Schiffer et al. (2009) suggest, however, that continued tLED treatments, would seem to be necessary.

4.4 PTSD, and Relationship to resting state, functional connectivity MRI networks (DMN and SN)

Impaired response inhibition has been observed in veterans who had mTBI plus PTSD (or mTBI without PTSD) [113]. These authors observed more errors on NoGo trials in both groups, compared to controls. In the Naeser et al., (2014) study, four of the mTBI cases had PCL-C scores pre- tLED, that were suggestive of PTSD and 3 of these 4 cases also had pre- tLED, Stroop Trial 4, Inhibition Switching scores that were at least -2 SD at entry (P4, 5, 10). (The fourth case, P3, entered with an average score of 0 SD.) All four of these cases reported reduced PTSD symptoms by a "reliable decrease," or a "clinically meaningful decrease," Post- tLED (Fig. 2) and all four of these cases improved by +1 SD to +2 SD on the Stroop Trial 4, Inhibition Switching, Post- tLED. Thus, improved inhibition could also have had a beneficial effect on level of PTSD, post- tLED.

Abnormalities in the DMN have been reported in studies with patients who have PTSD [114, 115]. Sripada and colleagues observed "reduced functional connectivity within the DMN (between DMN seeds and other DMN regions) including rostral ACC/vmPFC; and increased connectivity within the SN (between insula seeds and other SN regions) including the amygdala." In addition, there was an increased cross-network connectivity where DMN seeds showed elevated connectivity with SN regions including the insula; and SN seeds exhibited increased connectivity with DMN regions including the hippocampus. Their results suggested a dominance of "threat-sensitive circuitry in PTSD, even in task-free conditions. Disequilibrium between large-scale networks subserving salience detection versus internally focused thought may be associated with PTSD pathophysiology." Thus, the four participants who experienced reduced PTSD symptoms post- tLED therapy that included treatment of nodes within the DMN and the SN, may have experienced less PTSD symptoms due to better modulation between these two important intrinsic networks. Studies with rs-fMRI pre- and post- tLED, would be necessary to further examine the potential relationship between decrease in PTSD and LED placements.

Menon (2011) has proposed a "triple network model of aberrant saliency mapping and cognitive dysfunction in psychopathology," that is present in psychiatric as well as neurological disorders. The three large-scale brain networks are the DMN, the SN, and CEN. Results from the Naeser et al. (2014) tLED study with chronic, mTBI cases suggest that

all three of these networks may have been abnormal pre-LED, but modulated in function, post- a series of 18 tLED treatments that likely targeted nodes within each of these three networks. Stevens, Lovejoy, Kim et al., (2012) studied 12 separate resting state networks in 30 mTBI cases (13 to 136 days post- injury), and observed abnormal functional connectivity in every brain network, including visual processing, motor, limbic, and numerous circuits believed to underlie executive cognition; some connections were decreased, and some increased. "Postconcussive symptom severity was linked to abnormal regional connectivity within nearly every brain network identified, *particularly the anterior cingulate*." Thus, one of the most important tLED placement areas may be the LED placement area located at the midline of the face, centered over the front hairline and the forehead, likely targeting the dACC, of the SN; and the vmPFC, of the DMN. The LED placement area targeting the precuneus (DMN), and the LED placement areas targeting the DLPFC areas (CEN) are also among the most important placement areas.

4. 5. Weak connections between nodes within resting state functional connectivity networks in TBI

Several rs-fMRI studies with TBI patients have suggested that the intrinsic networks continue to be present post- injury, but their *connections are weak* [70, 117-119]. Some of the aberrant, or weak, functional connections may persist for months, or even years post- injury [120]. Menon (2011) has commented that weak anatomical connectivity within- and across-network nodes can compromise dynamic interaction of the core networks, all of which can result in abnormal psychiatric and neurological behavior.

4.6 Mechanisms and Cellular Effects, post- red/NIR tLED

While the specific mechanisms and cellular effects involved with improved cognitive function post- tLED are not entirely known, a few of possible mechanisms are listed below.

1. There may be increased ATP, especially in cells comprising the large-scale, intrinsic networks (SN, DMN, CEN), where large demands for energy are constant. Increased ATP would improve cellular respiration, oxygenation and function of these hypoxic/comprised cells.

2. There may be increased vasodilation/regional cerebral blood flow (rCBF) in surface cortical areas, subjacent to the scalp placements of the LED cluster heads. Previous animal studies using transcranial NIR LED to treat acute TBI [121] have suggested that the mitochondria are the primary target, where absorption of photons by cytochrome c oxidase releases bound nitric oxide (diffusing it outside the cell wall, promoting local vasodilation). Two previous studies with humans have reported an increase of rCBF in cortical areas, subjacent to LED placements on the forehead. [11, 12]

3. There is potential increase in anti-oxidants. The red/NIR photons induce redox-sensitive transcription factors such as nuclear factor-kappa B that promote gene transcription. The strong anti-oxidant, mitochondrial superoxide dismutase, is one of the most upregulated genes after NF-kB activation. [122] Another highly upregulated gene after NF-kB activation and after LED/LLLT is heat-shock protein 70, a molecular chaperone for protein molecules that prevents mis-folding and unwanted protein aggregation, especially at the telomeres of the DNA. [123]

4. There may be decreased inflammation, post- tLED. Photons in the red/NIR wavelengths reduce inflammation. [124] The study by Khuman et al. (2012) showed that transcranial, NIR LLLT therapy had an anti-inflammatory effect, inhibiting microglial activation, when treating acute TBI in mice. Many reports [125] demonstrate that red/NIR photons reduce COX-2 expression levels and reduce prostaglandins in multiple animal models, as well as in vitro. [126, 127]

5. There may be increased sleep, which was observed in a quantitative manner using the Actiwatch system, in our pilot studies at the VA Boston Healthcare System. The improved sleep was observed in TBI cases who received the tLED treatment series, [105] as well as the single case who received the intranasal LED treatment series. [128]

6 and 7. These may be the most important, as the tLED therapies are hypothesized to improve brain function by stimulating *neurogenesis* and *synaptogenesis*. These notions are based on small animal studies using tLLLT to treat acute, severe TBI in mice. [19, 20] Two key sites for adult human neurogenesis include the subventricular zone of the lateral ventricles, and the subgranular layer of the dentate gyrus in the hippocampus. [129] "Neurogenesis persists in the adult mammalian brain, where it can be stimulated by physiological factors, such as growth factors and environmental enrichment, and by pathological processes, including ischemia…" [130]

5. CONCLUSIONS AND FUTURE STUDIES

Randomized, sham-controlled studies that can build on these promising results, appear warranted – both in acute and chronic TBI. If these behavioral results are replicated, and supportive rs-fMRI, task-oriented fMRI, and DTI studies are available, then perhaps additional patient populations (e.g., mild cognitive impairment or early Alzheimer's Disease) could be treated with red/NIR tLED therapy and/or intranasal LEDs (especially in the earlier stages of a progressive

disorder). In addition, the possibility of prevention of long-term cognitive dysfunction in TBI cases may be an option, if treatments can be provided earlier post- injury.

New pilot studies with tLED are in the beginning stages, at the VA Boston Healthcare System. Dr. Jeffrey Knight at the National Center for PTSD there, has obtained VA funding to explore real vs. sham tLED and intranasal LED to reduce symptoms of PTSD and improve cognition in veterans with TBI. Dr. Yelena Bogdanova has obtained VA funding to explore real vs. sham tLED and intranasal LED to improve sleep and cognition in veterans who have experienced blast TBI. Dr. Margaret Naeser has received VA funding to explore these modalities to improve cognition in veterans who have Gulf War Illness. Dr. Carole Palumbo has received funding from the Army Medical Department Advanced Medical Technology Initiative to test the validity of tLED and intranasal LED in active duty soldiers who have suffered blast TBI. Dr. Palumbo is an investigator at the US Army Institute of Environmental Medicine, at the VA Boston Healthcare System and Boston University School of Medicine.