Transcranial Light-Emitting Diode (LED) Therapy to Improve Cognition in GWVI
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<u>**Research Plan.**</u>

1. Rationale 1a. Statement of the Problem

Impaired cognition is one of the 3 major problem areas of Gulf War Veterans' Illnesses (GWVI) needing empirically-supported treatments. This sham-controlled study proposes to test the treatment effectiveness of red and near-infrared (NIR) light, delivered via painless, non-invasive, light-emitting diodes (LED), for **improving cognitive symptoms in the areas of Attention, Executive Function, and Memory** in GWVI veterans. Our pilot data show that red/NIR LED therapy effectively **reduces cognitive problems** - e.g., **Executive Function** (decision-making, multi-tasking, processing speed) and **Verbal Memory--** in chronic, traumatic brain injury (TBI; even 7 years post- TBI). **Increased regional cerebral blood flow (rCBF)** may underlie these effects. We provide pilot data showing **increased rCBF on functional MRI scans**, following a series of transcranial, red/NIR LED treatments of chronic stroke, and of neurodegenerative disease (Primary Progressive Aphasia, similar to Alzheimer's Disease). Significantly improved behavior was present post- LED. The **increase in rCBF** (vasodilation) is triggered by **release of nitric oxide from hypoxic/compromised cells, after exposure to the red/NIR photons.** It has been known since 1981, that approximately 3% of NIR photons applied at the scalp can penetrate 1cm deep, to reach brain cortex. In hypoxic/compromised cells, cytochrome C oxidase (the last complex in the electron transport chain) is saturated with nitric oxide; and there is little adenosine tri-phosphate (ATP) production to support needed energy for cellular function. Cytochrome C oxidase is a photo-acceptor for red/NIR wavelengths. After exposure to the red/NIR photons, the **nitric oxide is diffused outside the cell wall** where it promotes an increase in vasodilation. At the same time, cytochrome C oxidase **increases ATP production in the mitochondria**. The cellular effects last beyond the exposure to the red/NIR photons (Lane, 2006). This is referred to as "photobiomodulation."

This second major benefit, **improved mitochondrial function with increased ATP production,** is particularly important for GWVI. Studies have reported the **presence of mitochondrial dysfunction in veterans with GWVI.** The mitochondrial dysfunction is associated with neurotoxicant exposures during deployment (Golomb, 2008). These include organophosphate pesticides (OP); and pretreatment nerve agents, pyridostigmine bromide (PB) pills (Abu-Qare, Abou-Donia, 2001). Exposures to OP and PB have been shown to result in **tissue loss in the central nervous system** from **direct insults to mitochondria** (Kaur et al., 2007), and resultant cellular damage from myelin and neuronal breakdown products (Heaton et al., 2007).

Other benefits from treatment of hypoxic/compromised cells with red/NIR photons include decrease in inflammation, and an increase in angiogenesis, neurogenesis and synaptogenesis. We provide laboratory data from one of our consultants (M. Hamblin, PhD, Cell Biologist, Wellman Center for Photomedicine, MGH), showing an **increase in brain-derived neurotrophic factor (BDNF)** in animal studies post- TBI, when treated with transcranial NIR photons. BDNF is a major regulator of the formation of new neurons, and connections between neurons. His cell studies involved particularly the hippocampus. **GWI veterans** have **reduced size of the hippocampus** (Chao et al., 2010), a brain area important in forming new memories. A significant increase in Verbal Memory was observed in our chronic TBI cases post- red/NIR transcranial LED. We expect to observe similar improvements in GWVI veterans treated with transcranial, red/NIR LED.

1b. Hypotheses

1) After 15 **Sham** LED treatments, there will be <u>no significant improvement</u> in the cognitive measures, as tested within 1 week post- the 15th Sham LED treatment.

2) After 15 **Real** LED treatments, there will be <u>significant improvement</u> in the cognitive measures, as tested within 1 week post- the 15th Real LED treatment. (Results from 1 and 2 will provide efficacy-of-treatment data.)

3) There will be maintenance of improved cognition at 2 Mo. post- the 15th Real LED treatment.

1c. Specific Objectives

<u>Aim 1:</u> To significantly improve cognition in veterans with GWVI who have cognitive dysfunction, using transcranial, red/NIR LED. The cognitive tests are the Primary Outcome Measures.

<u>Aim 2:</u> To study the indirect effects of transcranial, red/NIR LED on other aspects of GWVI, including pain, fatigue, and mood. Potential biomarkers in the blood will also be examined (e.g., blood tests for mitochondrial function, inflammation, and coagulation), pre- and post- Sham and Real LED treatments. These are Secondary Outcome Measures, and are expected to provide directions for future studies.

1d. Expected Outcomes and Endpoints

The primary endpoint is to have data showing that transcranial, red/NIR LED significantly improves cognitive function in veterans with GWVI.

1e. Impact of Results from the Proposed Research to the VA, and to the Field of GWVI Over 200,000 Gulf War Veterans have developed the multi-symptom illness of GWVI. This will be the **first study** to investigate a science- and evidence-based treatment expected ***to improve cognition*** in veterans with GWVI.

2a. Background
2a. 1. Background
2a. 1a. i. Gulf War Veterans' Illnesses

Approximately 25-30% of the 697,000 Gulf War deployed veterans continue to report persistent multi-symptom illness (Steele, 2000; RAC, 2008). The Kansas City case definition (Steele, 2000) and Fukuda et al., (1998) have characterized Gulf War Veterans' Illnesses (GWVI) as 1 or more chronic symptoms (lasting >6 months) from at least 2 of 3 symptom categories: 1) musculoskeletal (muscle pain, or joint pain, stiffness); 2) mood-cognition; 3) fatigue. Headaches and/or GI problems may also be present. **To date, there are no successful treatments to improve cognitive function in GVWI.** Treating the mechanistic-based cause of GWVI in the 175,000 – 209,000 ill Gulf War veterans, remains a top priority for government agencies and advisory panels (RAC, 2008).

This proposal will address treating one potential underlying basis for GWVI - e.g., **mitochondrial dysfunction,** in brain neurons and white matter. This will **improve cerebral blood flow**, and thus **treat one of the major symptoms** of GWVI - e.g., **cognitive dysfunction**.

Transcranial, light-emitting diodes (LED) in the red and near-infrared (NIR) wavelengths, will be used. We later present pilot data for **improved cognitive function** post- transcranial, red/NIR LED therapy in cases with chronic, traumatic brain injury.

It has been hypothesized that following the First Gulf War, **mitochondrial dysfunction** is present in veterans who present with multi-symptom illness. Mitochondrial dysfunction may be located in the *brain* (associated with cognitive dysfunction); and in the muscles (associated with pain and fatigue) (Sullivan, Krengel et al., 2003; Golomb, 2007 and 2008; GWI RAC, 2008).

Evidence that **oxidative stress and mitochondrial dysfunction** have a role in GWVI was recently provided in a report by Golomb (2011), where the effectiveness of the supplement, coenzyme Q10, an antioxidant that plays a role in cellular production of adenosine tri-phosphate (ATP), was investigated. Results showed significant improvement across the most common symptoms in GWI for coenzyme Q10 vs. placebo (p=0.015 (Golomb, GWI RAC Presentation, 2011). For the 20 top-reported symptoms (those in 50% of subjects), each symptom showed a favorable direction difference for coenzyme Q10 vs. placebo (p<0.001). Symptoms that improved included headache, attention problems, diarrhea, energy to do things, impatience, tiredness, and dry skin. There was a trend-to-benefit for anxiety, tinnitus, memory/recall, reading comprehension, and sleep problems. On a 5 point self-rated health scale (poor, fair, good, very good, excellent) there was also a trend-to-benefit that increased from a baseline mean of 2.2 "fair," up 0.7, "good," (p=0.1). Systolic blood pressure (SBP) benefited whether low or high. Low SBP showed a 7 point rise; vs. 6 point further drop on placebo (13 point benefit = significant). High SBP showed a 23 point drop vs. 13 point drop on placebo (10 point benefit = trend). There was a benefit in Physical Function (SPS) (p=0.05), where 73% of those in the Q10 group improved, and 0% were worse. Among those in the Placebo group, 35% improved, and 15% were worse. *Dr. Golomb is a consultant on this proposed LED research project.*

One potential **cause for the mitochondrial dysfunction** is **neurotoxicant exposures** during deployment. These include organophosphate pesticides (OP); and pretreatment nerve agents, pyridostigmine bromide (PB) pills (Abu-Qare, Abou-Donia, 2001). Exposures to OP and PB have been shown to result in **tissue loss in the central nervous system from direct insults to mitochondria** (Kaur et al., 2007), and resultant cellular damage from myelin and neuronal breakdown products (Heaton et al., 2007). Cellular debris activates the immune cells of the CNS by glial activation (Milligan and Watkins, 2003, Rivest, 2009). Microglia are considered the immune cells of the CNS. Glial activation from foreign invaders to the CNS result in release of pro-inflammatory cytokines (IL-1, IL-6, TNF) that result in symptoms including cognitive difficulties, muscle and joint pain, and fatigue (Watkins et al., 2008; Fields, 2009).

2a. 1a. ii. Brain Imaging Studies, Brain Abnormalities and Cognitive Dysfunction in GWVI

Brain imaging studies have observed an association between **cognitive dysfunction** and **brain abnormalities in veterans with GWVI**. Heaton, et al., (2007) reported that linear trend analyses showed a significant association between higher levels of estimated sarin/cyclosarin exposure and reduced white matter (WM), and increased right lateral ventricle and increased left lateral ventricle volumes. Their findings suggest a subtle but persistent **central nervous system pathology** in GW veterans potentially exposed to even low

levels of sarin/cyclosarin. In another structural MRI study with GW veterans who also have PTSD, the **head of the hippocampus** was observed to be **significantly smaller**, than in healthy civilians (Vythilingam, et al., 2005).

Liu et al., (2011) reported **significant decreases in cerebral blood flow (CBF)** in deep brain structures (**amygdala, hippocampus** and **caudate**) in GWI cases **with cognitive dysfunction**. Their study utilized fMRI with an infusion of physostigmine, as an inhibitory cholinergic challenge. The rationale to use physostigmine as an inhibitory cholinergic challenge was based on the notion that **neural damage** and **cognitive deficits** are associated with excessive exposure to cholinesterase-inhibiting cholinergic stimulants – e.g., OP, PB, and low-level sarin nerve gas in fallout from Coalition bombing of Iraqi ammunition storage sites (RAC, 2008; Golomb, 2008; Henderson et al., 2001). These results with fMRI were similar to their results obtained earlier with SPECT brain scans, in the same veterans with GWI and cognitive dysfunction (Haley et al., 2009).

Chao et al., (2010) reported that in studying 40 GW-deployed veterans exposed to sarin/cyclosarin, there was significantly **reduced total gray matter** (GM) and **hippocampal volumes** (compared to 40 matched controls). There was a significant positive correlation between **total WM volume** and measures of **executive function** and **visuospatial abilities** in veterans with suspected sarin/cyclosarin exposure. In a later study, with 64 different veterans exposed to sarin/cyclosarin, binary comparisons revealed **significantly reduced GM and WM volumes** in the exposed veterans (compared to controls). On the Continuous Performance Test (CPT), a **measure of sustained** and **selective attention**, the exposed veterans had **significantly more errors of omission** and tended to have **slower responses**. There was an effect of GWI/Chronic Multisymptom Illness on both GM and WM volume in the exposed veterans (Chao et al, 2011).

Blood tests may allow biomarkers of mitochondrial damage and neuro-inflammation to be measured. OP exposures have been shown to affect indices of mitochondrial health such as lactate and pyruvate (Abu-Qare, Abou-Donia, 2001). In addition, chronic neuro-inflammation may be examined by IL-1, IL-6, TNF in the blood. Blood tests with GWI veterans have shown a state of hypercoagulation in some cases (Hannan et al., 2000).

Effective treatments for the symptoms of GWVI have remained elusive. A recent controlled study, however, using nasal, continuous positive airway pressure (CPAP), has shown that when the real CPAP mask was used at night in cases with sleep disordered breathing, the sleep significantly improved, as well as pain, fatigue, cognitive function, physical health and mental health (Amin et al., 2011). These results are encouraging, because they suggest that new treatments may be effective to help treat veterans with GWVI.

> This proposal will investigate whether the new treatment, transcranial, light-emitting diodes (LED) in the red/near-infrared wavelengths, can improve cognition in veterans with GWVI. Later, we provide **pilot data** with chronic, mild TBI cases **showing significant improvement in Executive Function** and **Memory**, post- LED.

2a. 1b. Introduction to Effects of Light-emitting Diode (LED) Therapy
Scalp application of red and near-infrared (NIR) light/photons, utilizing light-emitting diodes (LED), is a new application of LED technology. It has been known since the early 1980's, that approximately 3% of NIR photons applied to the scalp, can penetrate 1 cm deep, to reach brain cortex (Wan et al. 1981). **Vasodilation** and **increased regional cerebral blood flow** (rCBF) at the **targeted cortical areas** have been observed following application of NIR light/photons to the head, along with improved behavior in these human studies (Schiffer et al., 2009; Nawashiro et al., 2011). We also provide **pilot data** in this proposal, showing **increase in rCBF** post- a series of LED treatments in chronic stroke, and in neurodegenerative disease. **The effect is non-thermal**.

After exposure to red/NIR photons, there is **improved mitochondrial function** - improving cellular respiration, oxygenation, and cell function (Eells et al., 2003; Karu et al., 2005; Wong-Riley et al., 2005). The putative mechanism by which the improved mitochondrial function occurs is as follows: Photons in the red and NIR wavelengths are absorbed by cytochrome C oxidase (e.g., a photo-acceptor). Cytochrome c oxidase is the last complex in the electron transport chain located within the inner mitochondrial membrane (Karu et al., 1995; Eells et al., 2003; Wong-Riley et al., 2005). In hypoxic/poorly functioning cells, cytochrome C oxidase is hypothesized to be saturated with nitric oxide. When the mitochondria (including cytochrome C oxidase) are exposed to red/NIR photons, at least two major events occur:

1) The **nitric oxide is diffused outside the cell wall**, where this **promotes vasodilation** and **increased rCBF**.

2) The cytochrome C oxidase **increases adenosine tri-phosphate (ATP) production**, and **improves** cellular respiration, oxygenation, and **cell function.**

Two published studies have now shown **increased rCBF,** post- **transcranial, near-infrared (NIR) LED treatments in humans**. These are reviewed briefly, below:

Nawashiro et al., 2011: A severe TBI patient (persistent vegetative state for 7 mo.) was treated with transcranial, NIR LED for 3 Mo. A NIR LED cluster head was applied to left (L) and right (R) forehead for 30 min per LED session (850 nm wavelength, power density, 11.4mW/cm^2; energy density, 20.5 J/cm^2 at the skin). Post- LED therapy, the patient showed improv ement in his neurological condition by moving his L arm/hand to reach the tracheostomy tube; SPECT scan showed increased **rCBF** in **L frontal lobe**. Fig.1.
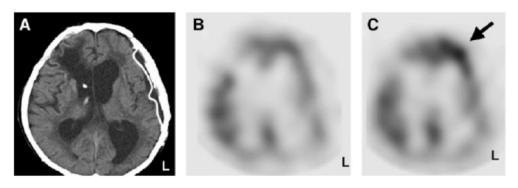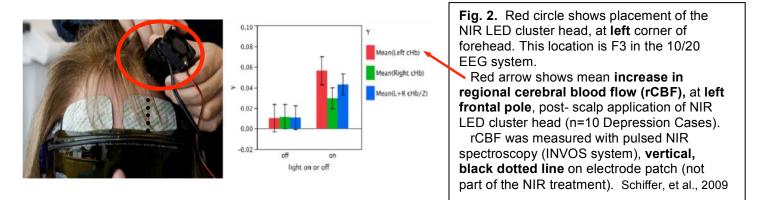

Fig. 1. A) CT scan for persistent vegetative state case, at 7 Mo. post-severe TBI. **B)** SPECT scan also at 7 Mo.; Pre- transcranial LED therapy. **C)** SPECT scan at 30 min after last LED treatment, after 3 Mo. of LED therapy, showing **focal increase of 20%** (vs. Pre-LED) for **rCBF** in the **left anterior frontal cortex** (black arrow).   Nawashiro et al., 2011

Schiffer et al., 2009: **Increased rCBF in** the **L and R frontal pole** regions was observed immediately after application of NIR LED to the forehead in 10 chronic, severe depression cases (Fig. 2). There was **significant reduction in depression and anxiety** for 2 weeks, following the single LED treatment (Fig. 3). The LED cluster head was applied for 4 min each, at the F3 and F4 locations based on the 10/20 EEG system (810 nm; power density, 250 mW/cm^2 at 4 mm from skin; 60 J/cm^2, estimated 2.1 J/cm^2 to two treated areas of brain).

Fig. 2. Red circle shows placement of the NIR LED cluster head, at **left** corner of forehead. This location is F3 in the 10/20 EEG system.
  Red arrow shows mean **increase in regional cerebral blood flow (rCBF),** at **left frontal pole**, post- scalp application of NIR LED cluster head (n=10 Depression Cases).
  rCBF was measured with pulsed NIR spectroscopy (INVOS system), **vertical, black dotted line** on electrode patch (not part of the NIR treatment). Schiffer, et al., 2009
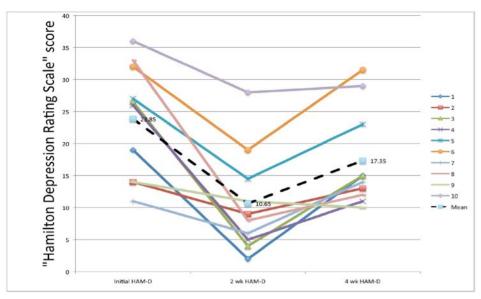
Fig. 3. Significantly (p=.001) **reduced Depression scores** (Hamilton D Rating Scale) at 2 weeks after one transcranial, NIR LED treatment to F3 and F4 frontal areas (10/20 EEG system) for 4 minutes to each area, in 10 severe, chronic depression cases. See Fig. 2.

A high score suggests more depression. Fifteen or above is suggestive of a clinical depression and below 8 is suggestive of a remission. The legend numbers correspond to patient numbers.
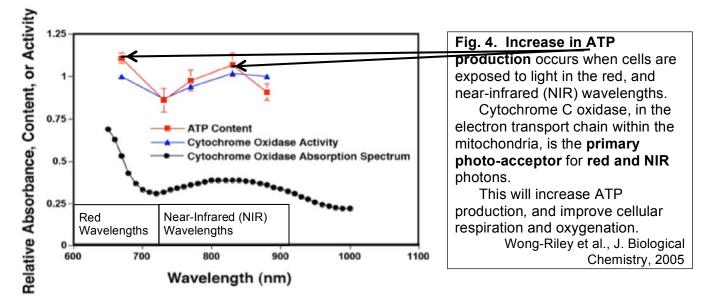
Schiffer et al., 2009

In pilot studies, the **PI and colleagues** have also observed **increased rCBF** on **functional MRI scans** (fMRI) in chronic stroke, and in neurodegenerative disease, post- transcranial LED treatments, compared to pre- LED. Our pilot data fMRI scans are shown in **Section 2a. 2a. i - iv, Preliminary Studies by the PI**.
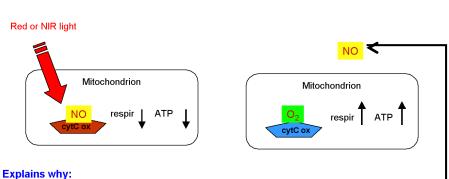
The transcranial LED intervention is expected to **improve brain-related functioning (cognition),** by **increasing rCBF in targeted cortical areas in the brain;** and **increasing ATP.** LED therapy **is expected to improve cellular activity particularly** in hypoxic/hypometabolic cells that are present throughout the brain in GW veterans with cognitive dysfunction. Our **fMRI pilot data support a focal increase in rCBF, post- LED.**

2a. 1c. Cellular Effects of Red, and Near-infrared (NIR) Photons

The technology of low-level laser therapy (LLLT) that includes non-thermal lasers (less than 500 mW), or even non-coherent light emitting diodes (LEDs) in the red or near-infrared (NIR) wavelength range, is commonly known as cold laser, biostimulation or **photobiomodulation**. All types (coherent lasers or non-coherent LEDs) have been shown to produce beneficial cellular effects in controlled trials (Desmet et al. 2006, review). During LED/LLLT, absorption of red or NIR photons by cytochrome C oxidase in the mitochondrial respiratory chain (Lane, 2006) causes an **increase in cellular respiration that continues for much longer than the light is present** when delivered at appropriate fluence and exposure durations. The primary cellular effect includes an **increase in mitochondrial activity** and an **increase in ATP** levels (Karu et al., 1995; Eells et al., 2003; Wong-Riley et al., 2005). See Fig. 4.

Fig. 4. Increase in ATP production occurs when cells are exposed to light in the red, and near-infrared (NIR) wavelengths.

Cytochrome C oxidase, in the electron transport chain within the mitochondria, is the **primary photo-acceptor** for **red and NIR** photons.

This will increase ATP production, and improve cellular respiration and oxygenation.

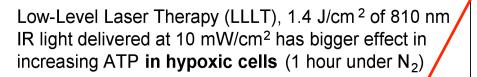Wong-Riley et al., J. Biological Chemistry, 2005

When the oxygen concentration is low, nanomolar concentrations of **nitric oxide (NO) can effectively act as a regulator of the mitochondrial respiratory chain**. The central hypothesis is that red and NIR photons that are specifically absorbed by cytochrome C oxidase, disrupt the weak non-covalent bonds between NO and its two binding sites in cytochrome C oxidase, allowing the NO molecule to diffuse away (Karu, Pyatibrat, Afanasyeva, 2005; Lane, 2006). Fig. 5. Thus, this primary photon absorption event in the mitochondria leads to NO release, that is responsible for the increased blood flow that is measured in the brain shortly after illumination, Fig. 2 (Schiffer et al., 2009).

Red or NIR light

Mitochondrion
NO
cytC ox
respir ↓ ATP ↓

Mitochondrion
O_2
cytC ox
respir ↑ ATP ↑

NO

Explains why:
Normal cells and tissue generally do not respond
Hypoxic cells, damaged cells, and tissue at risk of death respond well
Effects continue for long time after light is switched off
Released nitric oxide temporarily increases blood flow in illuminated area
Released nitric oxide reduces swelling by dilating   lymphatics and increasing drainage

Fig. 5. Hypothesis regarding effect of red or near-infrared (NIR) light on the primary photo-acceptor, cytochrome C oxidase (cytC ox, in this diagram), located in the mitochondrial membrane. Before red/NIR LLLT/LED application, the CytC ox appears "saturated" with nitric oxide (NO). After application of red/NIR photons, the **mitochondria increase ATP production** and improve respiration.
    The **NO is diffused outside the cell wall**, and this **promotes vasodilation.** Our fMRI pilot data support this, see Sect. 2a. 2b. i.
            Hamblin; Wellman Center for Photomedicine, Mass.General Hospital

Since nitric oxide (NO) competes with oxygen for binding to cytC ox, the release of NO will have a greater effect, and a **long-lasting effect on respiration in *hypoxic* cells**, compared to normoxic cells. See Fig. 6.
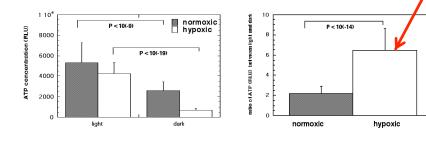
Fig. 6. NIR photons promote a **greater increase of ATP in *hypoxic* cells** (6x increase), than in normoxic cells (2x increase). Mean luminescence values (+/- SD) from 12 wells of HeLa cells (a human cervical cancer cell line used to demonstrate LLLT effects) treated or not, with 1.4 J/cm^2 of NIR 810 nm light in either normoxic conditions (regular atmosphere) or hypoxic conditions (1 hour exposure to pure nitrogen after 3 cycles of vacuum).
  M. Hamblin, PhD, Consultant on this project;
      Wellman Center for Photomedicine, MGH

Low-Level Laser Therapy (LLLT), 1.4 J/cm^2 of 810 nm IR light delivered at 10 mW/cm^2 has bigger effect in increasing ATP **in hypoxic cells** (1 hour under N_2)

2a. 1d. Promotion of Angiogenesis, Neurogenesis, and Synaptogenesis, from Red/NIR Photons

Neurogenesis is the generation of neuronal precursors and birth of new neural cells. Two key sites for adult neurogenesis include the subventricular zone (SVZ) of the lateral ventricles, and the subgranular layer (SGL) of the dentate gyrus in the hippocampus (Eriksson et al., 1998). "**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**…" (Jin et al., 2006).

Increased vascular endothelial growth factor **(VEGF)** has been associated with enhanced post-ischemic **neurogenesis and neuromigration**, in VEGF-over-expressing transgenic mice, consistent with a possible role in ischemic brain tissue repair (Wang, Jin, Mao et al., 2007). **VEGF** could provide a **therapeutic target** for more chronic **brain repair**. **NIR** laser therapy has been observed **to increase VEGF** and **angiogenesis** following myocardial infarction in animal studies (Tuby et al., 2006). **Scalp application of red/NIR LED could stimulate secretion of VEGF,** thus **promoting angiogenesis** and **neurogenesis.**

2a. 1e. Increase in Brain-Derived Neurotrophic Factor (BDNF), from Red/NIR Photons

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that is a **major regulator** of both **synaptic transmission**, and **plasticity** at adult synapses in the central nervous system (Kaplan, Vasterling, Vedek, 2010; Kim, Lee & Kim, 2010). **BDNF** has been observed to **increase survival of neurons**, **increase synaptic transmission** (Lipsky and Marini, 2007), long-term potentiation and depression, and certain forms of short-term synaptic plasticity (Desai et al., 1999). Thus, **BDNF has implications for the formation of memories** in patients with **impaired memory** and **cognition** (Kaplan et al., 2010).

Recent animal studies at the Wellman Center for Photomedicine, Mass. General Hospital (M. Hamblin, PhD, personal communication, Consultant on this project) have observed **increased BDNF** expression in the **injured brain** (TBI), at **7 and 28 days after a single application of NIR photons** (transcranial, low-level laser therapy, 810-nm laser), given 4 hours post- TBI (see Figs. 7 and 8). **Increased neurogenesis** and **synaptogenesis** in the **hippocampus** was observed post- transcranial NIR, low-level laser therapy.
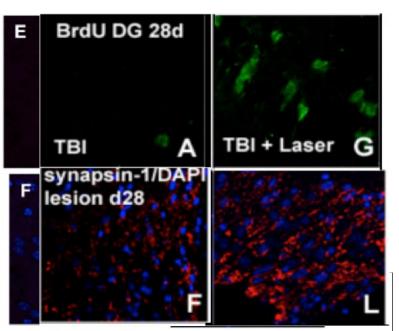
This finding suggests that **neurogenesis** has been stimulated in the subgranular zone (SGZ) of the dentate gyrus in the **hippocampus,** by **transcranial NIR photons.** It is unclear if the light actually needs to penetrate to the hippocampus, or **if hippocampal neurogenesis** can be **stimulated by BDNF** generated **in the cortex**, in response to exposure of the NIR photons applied outside the skull, transcranially.

These **increases in BDNF, neurogenesis and synaptogenesis** explain **why**

Fig. 7. Neurogenesis and **synaptogenesis** in a mouse model of TBI, that received a single NIR, 810-nm transcranial laser treatment (18 J/cm2, at 25 mW/cm2), at 4 hours post- TBI.

Day 7 (panels E, G) show **increased brain-derived neurotrophic factor (BDNF)** expression in the dentate gyrus of the **hippocampus** (pink, panel G) in the **TBI laser** sample. G indicates **increased proliferating neuroprogenitor cells**; vs. E, the sample not treated with NIR laser.

Day 7 (panels F, H) show an increase of DAPI (purple, panel H) in the TBI laser sample. H indicates increased **formation of new connections between existing neurons**, and **suggesting improved neural plasticity** in the **TBI laser sample**; vs. F, the sample not treated with NIR laser.   Hamblin, MGH Consultant on this project.

Day 28 (panels A, G) show BrdU incorporation (green) in the dentate gyrus of the **hippocampus.** The **TBI + Laser** sample G, indicates **increased proliferating neuroprogenitor cells**; vs. A, the sample not treated with NIR laser.

Day 28 (panels F, L) show an increase of synapsin-1 (red) in lesion area, indicating the **formation of new connections between existing neurons**, and **suggesting improved neural plasticity** in the **TBI + Laser sample** L; vs. F, the sample not treated with NIR laser.

This is **relevant to GWVI** because studies have shown **decreased size of the hippocampus in GW veterans with cognitive dysfunction**. *Transcranial NIR, LED could* *potentially improve hippocampal function.*

transcranial NIR can be **beneficial** in such a **variety of brain diseases,** where these processes have been shown to be crucial in mediating therapeutic effects (De Taboada et al., 2011). Positive results of transcranial, red/NIR photons from LED/LLLT, have now been observed in animal models of **TBI** (Ando et al., 2011; Khuman et al., 2011; Oron et al., 2007), in humans with **chronic TBI** (Naeser et al., 2011), in humans with **severe depression** (Schiffer et al., 2009), in humans with **acute stroke** (Lampl et al., 2007; Zivin et al., 2009), and in humans with **chronic stroke** (Naeser, pilot data shown in this proposal), and in **neurodegenerative disease** (Naeser, pilot data shown in this proposal, with Primary Progressive Aphasia).

2a. 1f. Anti-Inflammatory Effect, from Red/NIR Photons
Red/NIR photons delivered with LED/LLLT produce transient, low levels of reactive oxygen species (ROS) in mitochondria of illuminated cells, and these ROS cause NF-kB activation via the redox sensitive sensor enzyme protein kinase D1 (Storz, 2007). Cell research (M. Hamblin, PhD, MGH Consultant on this project) has observed that repeated NIR LED/LLLT treatments **promote decreased inflammation (less NF-kB).**

This is partially explained when one considers the role of genes that respond to ROS-induced activation of NF-kB, including **antioxidants** and **up-regulated genes** such as **superoxide dismutase, glutathione peroxidase,** and **heat shock protein 70** (Storz, 2007; Sompol et al., 2006; Zhang et al., 1994; Avni et al., 2005). The **mitochondrial superoxide dismutase** is one of the **most upregulated genes after NF-kB activation** (Sompol et al., 2006). 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** (Zhang et al., 1994).

Photons in the red/NIR wavelengths reduce oxidative damage and **reduce inflammation** (Rojas & Gonzalez-Lima, 2011, review paper). Many reports (Castano et al., 2007) demonstrate that **red/NIR photons reduce COX-2 expression levels** and **reduce prostaglandins** in multiple animal models as well as in vitro (Aimbire et al., 2005; Albertini et al., 2007; Sakurai et al., 2000).

Another **key inflammatory mediator** that has been implicated in cell pathogenesis is the **cytokine tumor necrosis factor alpha (TNFα)**. There are multiple reports showing that **red/NIR photons reduce TNFα** levels in arthritis (Aimbire et al., 2006). There has been a report (Leung et al., 2002) that in a rat stroke model, transcranial LLLT triggered the expression of TGF-β1.

2a. 1g. *Acute Stroke,* Transcranial Application of NIR Photons

2a. 1g. i. *Animal Studies:* Acute Stroke, Transcranial Application of NIR Photons
Transcranial, NIR low-level laser therapy (using NIR photons, 808 nm) has significantly improved recovery following ischemic stroke in rats, when treated once, at 24 hours post-stroke (Lampl, 2007; Oron et al., 2006). Stroke was induced in rats by two different methods (A) permanent occlusion of the middle cerebral artery through a craniotomy or (B) insertion of a filament. A near-infrared (NIR) Ga-As diode laser was used transcranially, to illuminate the hemisphere contralateral to the stroke at a power density of 7.5 mW/cm^2 to brain tissue (Oron et al., 2006).
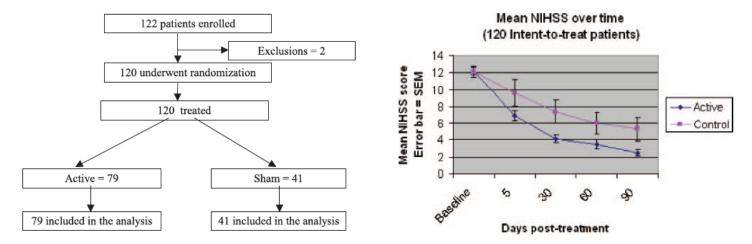
At 3 weeks poststroke, there was a **highly significant (p<.01) reduction** of 32% **in the neurological deficit** in the laser-treated rats, relative to control ones. The **number of newly formed neuronal cells**, assessed by double immunoreactivity to BrdU and tubulin isotype III, as well as migrating cells (doublecortin immunoactivity) was **significantly elevated in the subventricular zone (SVZ)** of the hemisphere **ipsilateral to the induction of stroke** when treated by LLLT (which had been applied to the contra-lesional side). There was no significant difference in the stroke lesion area between control and laser-irradiated rats. The authors suggested that an **underlying mechanism** for the **functional benefit** post- LLLT in their study was a possible **induction of neurogenesis**.
Other studies have also suggested that because improvement in neurologic outcome may not be evident for 2–4 weeks in the post-stroke rat model, **delayed benefits may be due**, in part, from **induction of neurogenesis** and **migration of neurons** (Shen et al., 2008; Detaboada et al., 2006). In addition, transcranial LLLT may prevent apoptosis and improve outcomes by exerting a neuroprotective effect, although these exact mechanisms are not well understood (Carnevalli et al., 2003).

Cortical ATP increase, post- NIR Low-Level Laser Therapy (LLLT) application in acute, embolized rabbits: A direct relationship between level of cortical fluence delivered (in J/cm^2) and **cortical ATP content** has been observed in a study with embolized rabbits (Lapchak and De Taboada, 2010). Five minutes following embolization (right carotid), rabbits were exposed to 2 minutes of transcranial NIR LLLT (continuous wave, CW; or pulsed wave, PW) on the skin surface, posterior to the bregma, at midline. Three hours after embolization, the cerebral cortex was excised, and processed for measurement of ATP content. Embolization had decreased cortical ATP content in ischemic cortex by 45%, compared to naive rabbits. **With** CW, **NIR** LLLT (**0.9 J/cm^2**; 7.5 mW/cm^2) there was an **increase of 41% in cortical ATP** in the embolized rabbits. With PW, **NIR** LLLT (100 Hz; **4.5 J/cm^2**; 37.5 mW/cm^2) there was an **increase of 157% in cortical ATP**. It was surprising that following transcranial NIR LLLT in the rabbits with stroke, the increased cortical ATP level of 157% was higher than that measured in naive rabbits that had never suffered stroke.

2a. 1g. ii. *Human Studies:* **Acute Stroke, Transcranial Application of NIR Photons**

Transcranial, NIR LLLT has been shown to **significantly improve outcome in human, acute stroke patients,** when applied at ~18 hours post-stroke, over the entire surface of the head (20 points in the 10/20 EEG system) regardless of stroke location (Lampl et al., 2007). Only one LLLT treatment was administered, and 5 days later, there was **significantly greater improvement in the Real-** but not in the Sham-treated group (p<.05, NIH Stroke Severity Scale). Fig. 9. This significantly greater improvement was still present at 90 days poststroke, where 70% of the patients treated with Real LLLT had successful outcome, only 51% of controls. A NIR (808 nm) laser was used, delivering a fluence of 0.9 J/cm^2 over the entire cortical surface (2 min. per each of the 20 points; power density of 7.5 mW/cm^2).

In a second, similar study with the same transcranial LLLT protocol, with an **additional 658 acute stroke**

Fig. 9. Significant improvement at day 5, after **1 Real, transcranial LLLT treatment** performed at ~**18 hours post-stroke** in acute human stroke patients (but not after 1 Sham LLLT Treatment). *Both hemispheres were treated*, regardless of side of stroke. *Energy dose to the brain cortex* was estimated to be only *1 Joule/cm^2*. No perceptible heat, Real or Sham. NeuroThera Laser System, PhotoThera Laser, San Diego. Class IV, near-infrared laser (>500 mW), 808 nm. **No adverse events.**   Lampl et al., 2007, *Stroke*

patients randomized for Real or Sham, transcranial LLLT, **similar significant beneficial results (p<.04)** were observed for those who received the **Real laser protocol**, for moderate and moderate-severe stroke patients (n=434) but not for severe patients (Zivin et al., 2009). When data for both stroke studies were pooled (n=778; including 120, Lampl et al., 2007; plus 658, Zivin et al., 2009), there was a **highly significant beneficial effect for the Real transcranial LLLT group (p=0.003),** versus those who received the Sham laser treatment (Stemer et al., 2010). There were **no negative side effects or adverse events.**

The present, proposed study will be **the first study to use transcranial red/NIR LED to improve cognition in veterans with GWVI.**

Transcranial, red/NIR LED **holds promise for treating other CNS-disorders** present in **veterans** – e.g., cognitive dysfunction from traumatic brain injury (TBI); chronic, post-traumatic stress disorder (PTSD); depression, anxiety; and neurodegenerative disorders - possibly in the early stages of Alzhiemer's Disease.

There are **no contraindications to transcranial LED therapy**, such as metallic implants or pacemakers.

Transcranial LED therapy also has potential for long-term, *home treatments*.

2a. 2. Preliminary Studies by the PI, using Transcranial, Red/NIR LED
We present pilot data with 3 patient populations, where we have used transcranial, LED to improve behavior:

i) **Chronic, traumatic brain injury (TBI)** cases, to improve cognition

ii) **Chronic,** (left-hemisphere) **stroke patients** who have aphasia, to improve language

iii) **Neurodegenerative disease** (Primary Progressive Aphasia), to improve language

We have observed significant improvement in cognition or language post- LED, in each patient type.

In addition, we have observed changes on functional MRI (fMRI) scans, pre- and post- transcranial, LED therapy, that parallel changes in language behavior. These fMRI studies show that **specific LED placement locations on the head** are associated with **specific effects on language behavior.**

Prior to our fMRI studies, this focal effect on brain cortex for NIR light applied outside the skull, was not known. Our results suggest a ***proof of principle*** for the effect of transcranial LED to affect neural networks, and improve behavior.

We also present resting state, ***functional connectivity MRI*** scan analyses following either **Sham LED**, or **Real LED** treatment, in the neurodegenerative disease, Primary Progressive Aphasia. **Only following the Real LED** treatment (at a dose of **13 J/cm2**, per LED placement), was there a **change in functional connectivity** between two, left frontal gyrus areas important for language (middle and inferior frontal gyrus).

We believe our pilot data provide a foundation, for a sham-controlled study examining whether transcranial, red/NIR LED therapy can improve cognitive function in veterans with GWVI, where cognitive dysfunction has been documented to be present.

Our pilot data with these 3 patient populations are summarized, below.

2a. 2a. ia. *Chronic, Traumatic Brain Injury:* Improved Cognition post- LED (published cases)
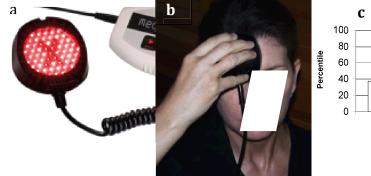The PI and colleagues have published two, chronic, mild traumatic brain injury (mTBI) cases where significant, **improved cognition** was observed after scalp application of red/NIR LED therapy (Naeser et al., 2011). Despite different etiologies, **veterans with GWVI share some similarities of patients with chronic TBI** - i.e., **hypoxic/hypometabolic cells** that are part of **neural networks for higher cortical functions**, and impaired behavior is present. **In chronic TBI** cases, for example, this results in poor performance **on attention** and **executive function tasks** (Kato et al., 2007; Sanchez-Carrion et al., 2008; Strangman et al., 2008; McAllister et al., 2006; Scheibel et al., 2007). **Poor performance on attention and executive function** is also **present in GW veterans with cognitive dysfunction**, as reviewed above, in section 2a. 1a. ii. "Brain Imaging Studies, Brain Abnormalities and Cognitive Dysfunction in GWVI" (Heaton et al., 2007; Haley et al., 2009; Liu et al., 2011; Chao et al., 2010; 2011). Our pilot studies with transcranial LED have observed **improved cognition, post- transcranial, red/NIR LED** treatments in **chronic TBI,** as reviewed below.
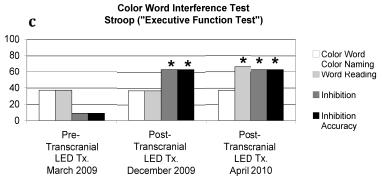
Published TBI case, P1 (66Yr F) began **transcranial, red/NIR LED treatments** at **7 years after closed-head mild TBI** (mTBI) from a car accident that occurred at age 59. For 7 years after the accident, and pre-transcranial, red/NIR LED therapy, her **sustained attention was limited to 20 minutes (computer work).** She had been a web designer, teaching at the university level. Post- 8 weeks of transcranial LED therapy, **her**

sustained attention increased to 3 hours (computer work). She has now **treated herself at home** nightly, for almost 7 years, with transcranial LED. She maintains her improved cognition, at age 73. There have been **no negative side effects**, and she reports improved sleep. She owns only 1, red/NIR LED cluster head that she uses at home (red/NIR, 500 mW). The cost of this FDA-cleared Home Treatment Device (1 LED cluster head) is $1,400 (manufactured by the MedX Health Co., Toronto).

 Published TBI case, P2 (52Yr F) with **mTBI+PTSD** is an **OIF veteran, retired high-ranking military officer** who had a history of multiple concussions during civilian traumas (rugby, sky diving), and military deployment. In 2007, she fell backwards from a swing, hitting the back of her head on concrete (with LOC, several minutes). Within 1 year, she required Medical Disability due to cognitive dysfunction. Fronto-parietal atrophy was present on her MRI scan (Naeser et al., 2011). There were significant problems with cognition, and modulating emotions and behavior (PTSD).

 Pre- transcranial LED, she had been on Medical Disability for 5 months. Post- 4 Mo. of transcranial LED treatments at home, she **returned to work full-time**. She was trained to perform LED treatments at home, using the MedX Health LED Device, Model 1100. This FDA-cleared device has 3 LED cluster heads (cost, $5,000). One of the LED cluster heads is shown in Fig. 10a,b.

Fig. 10a,b,c. Case **P2, mTBI+PTSD.** a.) Sample red and NIR, LED cluster head (2-inch diameter), for transcranial LED therapy, used in published TBI cases and in pilot LED studies. b) LED placement on R forehead, for one of the LED cluster heads. c) Graphs showing **significant improvement** in cognition, post- LED therapy in case, **P2 (OIF Veteran)** on tests of **Executive Function** (Inhibition, and Inhibition accuracy, +2 SD); and on the next page, **Memory** (**Immediate** and **Delayed Recall** +1,+2 SD). **She returned to full-employment, post- 4 Mo. of transcranial LED treatments at home.**
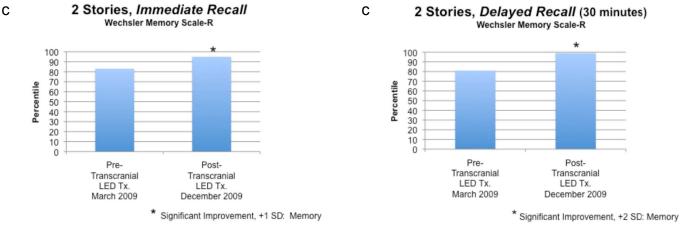

* +2 SD Improvement, inhibition, inhibition accuracy

 Neuropsychological testing **post-** 9 months of **transcranial LED therapy,** showed **significant improvement in cognition** - e.g., +2 SD, on the Stroop Test for **Executive Function** (Inhibition and Inhibition Accuracy); +1, and +2 SD on the Wechsler **Memory** Scale R, **Immediate and Delayed Recall**. See Fig. 11.

 Her **PTSD symptoms improved**, with **better emotional control in family, social and work settings.**

 After 4 months of nightly, transcranial **LED treatments at home**, **P2 returned to work full-time** (executive consultant, international technology); **she discontinued medical disability**.

Fig. 11. P2 cont'd. Significant Improvement in Memory, on Immediate and Delayed Recall, at 9 Mo. post- LED.

P2, Pre- and Post- LED Tx., *Neuropsychological Test Results*
Post- LED Testing,Post- 9 months, nightly, transcranial LED Tx.

c **2 Stories,** *Immediate Recall*
Wechsler Memory Scale-R

* Significant Improvement, +1 SD: Memory

P2, Pre- and Post- LED Tx., *Neuropsychological Test Results*
Post- LED Test, Post- 9 months, nightly, transcranial LED Tx.

c **2 Stories,** *Delayed Recall* (30 minutes)
Wechsler Memory Scale-R

* Significant Improvement, +2 SD: Memory

2a. 2a. i*b. Chronic, Traumatic Brain Injury:* **Improved Cognition post- LED** (7 Additional TBI Cases)
Pilot data for seven additional, chronic, mTBI cases (closed-head injury) who also showed significantly improved cognition, post- transcranial, red/NIR LED treatments are presented below. These 7 cases (non-veterans) were treated as part of a pilot, open-protocol study at Spaulding Rehabilitation Hospital, Boston, Harvard Medical School (personal communication, R. Zafonte, M. Naeser, M. Krengel, M. Hamblin, P. Martin).

They were treated with the same LED protocol described in Naeser et al., (2011), using the MedX Health LED cluster heads. Patients entered the study at 1-7 years post- closed-head, mTBI. To be eligible for enrollment, subjects scored at least 2 SD below the norm on one of the Neuropsychological (NP) cognitive tests, or 1 SD below the norm on at least two tests. These are well known, standardized NP tests: Trails A and B (Reynolds, 2002); FAS Test (Spreen & Benton, 1977); California Verbal Learning Test II (Delis et al., 2000); Stroop test for Executive Function (Delis, Kaplan, 2000).

Each case received 18 Real red/NIR LED, transcranial treatments; treated 3x per week (M,W,F) for 6 weeks, as an outpatient. Each LED cluster head had a 2-inch diameter, and was 500 mW; and contained 9 red LED diodes, and 52 NIR LED diodes; power density, 22.2 mW/cm2 (Fig. 10a). The LED cluster heads were placed bilaterally, on the forehead and scalp areas including frontal, temporal, parietal and occipital areas, and on the midline (front-to-back hairline) including at the vertex (10 min. per placement, two sets of placements, for a total of 20 min. of LED therapy at each visit). Each treatment visit lasted about 30-40 min. There were **no negative side effects** or complications. A few patients were sleepy after the initial LED treatments.

Patients received NP testing at Entry, and at 1 Wk., 1 Mo. and 2 Mo. after the 18th LED treatment.

Results for 7 Additional chronic, TBI Cases: There was **significantly improved cognition, on two tests –** e.g., **Executive Function**; and **Verbal Memory**, at 1 Wk. post- the 18th transcranial LED treatment (Figs. 12a,b, next page). Percentile scores were used for the pre- vs. post- LED data analyses, adjusting for age, gender and level of education. [Two patients have incomplete follow-up Testing at 1 Mo., and 2 Mo. post- LED therapy, thus complete statistical analyses could only be performed on the 1 Wk, post- LED data, at this time.]

On **Executive Function** (Stroop, Trial 4, Inhibition Accuracy), **significant improvement (p<.01** two-tail) was observed at the 1-Week post- LED testing. The pre- LED percentile mean was 28.9, SD=26.0; and the 1-Week post- LED percentile mean was 67.6, SD=35.6 (t=3.825). See Fig. 12a.

On **Verbal Memory**, California Verbal Learning Test (CVLT) II, Total Trials 1-5, **significant improvement (p<.05** two-tail) was also observed. The pre- LED percentile mean was 39.3, SD=26.9; and the 1-Week post- LED percentile mean was 58.1, SD=17.9 (t=2.434). See Fig. 12b.
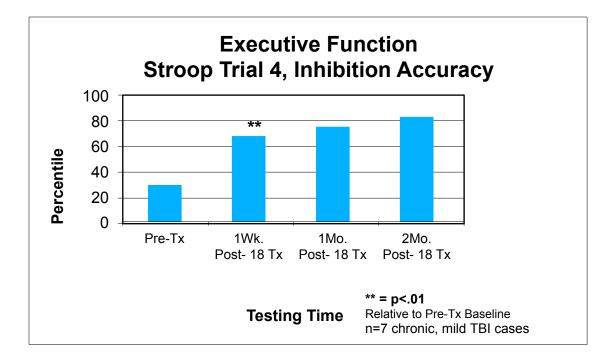
**Executive Function
Stroop Trial 4, Inhibition Accuracy**

Fig. 12a. Significant improvement (p<.01) on **Executive Function** for the Stroop Test (Trial 4, Inhibition Accuracy), tested at 1 Week, post- the 18th transcranial LED treatment. This test examines selective attention, cognitive flexibility, and processing speed. Pilot Data, n=7 chronic, **TBI cases**, pre- and post- transcranial, red/NIR LED treatments. Spaulding Rehabilitation Hospital, Boston.   Zafonte, Naeser, Krengel, Hamblin, Martin
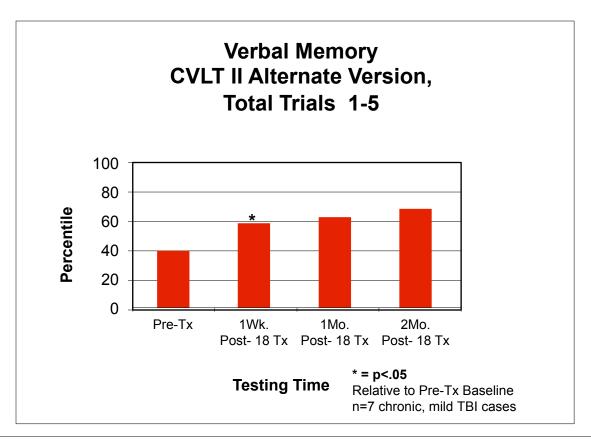

**Verbal Memory
CVLT II Alternate Version,
Total Trials 1-5**

Fig. 12b. Significant improvement (p<.05) on **Verbal Memory**, California Verbal Learning Test, tested at 1 Week, post- the 18th transcranial LED treatment. Pilot Data, n=7 chronic, **TBI cases**, pre- and post- transcranial, red/NIR LED treatments. Spaulding Rehabilitation Hospital, Boston.   Zafonte, Naeser, Krengel, Hamblin, Martin

Seven Additional TBI Cases, Demographics, and Medical History, <u>Spaulding Rehabilitation Hospital</u>

<u>P001, mTBI</u>: 52 Yr. F, at Entry (5 years post- TBI, MVA). College-educated, and high-ranking position in finance field, prior to TBI. On Medical Disability, 5 years, unable to return to work. Post- LED therapy, able to sort bills, write checks, read essays, **tasks previously unable to do for 5 years, since the TBI**.

<u>P004, mTBI</u>: 59 Yr. M, at Entry (2.5 years post- TBI, MVA). PhD degree, and continued to work part-time (22 hours/week after TBI, down from 30-34 hours/week before TBI). Post- LED therapy, his **headache pain was reduced from VAS of 5, down to VAS of 2**; and he no longer requires Extra Strength Tylenol or Tylenol, for HA pain. He continues to work.

<u>P005, mTBI</u>: 50 Yr. F, at Entry (20 months post-TBI, where she was a pedestrian running on sidewalk when hit by a car). MA degree, had taught English on the college level. She was depressed, and non-talkative at Entry, but became quite verbal and talkative after a few weeks of LED treatments. She continues to have right shoulder pain, and cannot return to work. Her husband reports that she is "better adjusted" at home.

<u>P006, mTBI+PTSD</u>: 26 Yr. F, at Entry (16 months post- TBI, four-car MVA, and hit by two cars, from behind her car). Two years of college. Unemployed. Also has PTSD post- MVA. **PTSD Checklist-Civilian was Severe (score of 47) at Entry,** and improved to **Mild (score of 30)** at **1 Week post- the 18th LED treatment**.

<u>P007, mTBI</u>: 58 Yr. M, at Entry (1 year post- TBI, hit in the head at close range, by a baseball that was hit by a bat). College educated, high school teacher and baseball coach. On Medical Disability. At 1 Wk, and at 1 Mo. post- the 18th LED treatment, he became "more active." He did taxes, and went on a job interview.

<u>P009, mTBI</u>: 62 Yr. F; 7 Yr. post- MVA. Lab tech, hair dresser; disabled.

<u>P011, mTBI</u>: 49 Yr. F; 3 Yr. post- TBI (hit by a snowboarder while standing still). He had a history of multiple concussions, and stroke at age 37. B.S. degree, hotel management; disabled.

In summary, our **pilot studies** with **transcranial, red/NIR LED** show **significant improvement** in **executive function** and **verbal memory,** post- LED treatments in chronic, TBI cases (Naeser et al., 2011, where n=2; and pilot data from Spaulding Rehabilitation Hospital, where n=7).

2a. 2a. ii. *Chronic Stroke Patients with Aphasia:* **Improved Language post- LED** (and fMRI scan data)

Despite different etiologies, **veterans with GWVI share some similarities of patients with chronic stroke** – i.e., **hypoxic/hypometabolic cells** that are part of **neural networks** for **higher cortical functions**, and impaired behavior is present. In chronic stroke patients with left-hemisphere stroke and aphasia, hypoxic/hypometabolic cells (in left, peri-lesional areas) are part of the remaining neural networks for language. This results in poor performance on language tasks (Naeser Martin et al., 2004; Martin, Naeser et al., 2005; 2009). Our pilot studies with transcranial, red/NIR LED show significant language improvement, post-transcranial, red/NIR LED in chronic stroke patients with aphasia.

Two chronic stroke patients, with left-hemisphere stroke and aphasia have been treated with transcranial LED (at 18, and at 12 years post-stroke). Both had nonfluent aphasia, as tested with the Boston Diagnostic Aphasia Exam (BDAE) (Goodglass, Kaplan, Barresi, 2001). They were both treated using two different LED Placement Paradigms. They were first treated with a **Bilateral, LED Placement Paradigm** during Summer 2011. This was the same as the original, transcranial LED protocol used with the chronic TBI cases (Naeser et al., 2011), and explained above, in Section 2a. 2a. "Transcranial LED to treat Chronic, Traumatic Brain Injury."

Later, these aphasia patients were treated with a **Left Hemisphere Only, LED Placement Paradigm**, during Fall 2011. We observed **different, significant language improvements** with each paradigm, and **different activation patterns on fMRI**, after treatment with each of the two LED Placement paradigms. Results for each of the two different LED Placement Paradigms are presented below.
<u>First Transcranial LED Treatment Method for Aphasia due to Stroke: *Bilateral LED Treatments*</u>

The same LED device that had been previously used with the TBI cases (Naeser et al., 2011; and Spaulding Rehabilitation Hospital, Boston), was used with the aphasia cases treated at the VA Boston Healthcare System (JP campus) – e.g., the MedX Health LED Console Model 1100 (Toronto). Each Model 1100, has three, 2-inch diameter, LED cluster heads. Two Model 1100 devices were used at the same time (6 cluster heads were in place on the head, at the same time). Each LED cluster head was 500 mW; and contained 9, red 633nm LED diodes; and 52, NIR 870nm LED diodes; the power density, 22.2 mW/cm2.

These are **non-thermal devices; there is no sensation of heat or pain**. It is an entirely painless, non-invasive treatment. The MedX Health LED device is **FDA-cleared, as non-significant risk**, and used to ...temporarily increase blood circulation for ...temporary decrease in musculoskeletal pain. The FDA-clearances date from 2003.

A dose of 13 Joules/cm^2 (12 min 11 sec) was applied to each of the bilateral scalp placement areas (L and R forehead areas, L and R perisylvian language areas, and midline LED placement areas including the R and L SMAs). The patients were treated in a soft recliner chair. The 6 LED cluster heads were held in place with a soft elastic cap, and Velcro bands were used if necessary, to secure the LEDs in place.

Each stroke patient received 18 transcranial LED treatments (M,W,F) for 6 weeks, with 13 J/cm^2 applied at each LED placement location using pulsed wave, 146 Hz (12 min 11 sec per LED placement). It was estimated that 0.4 J/cm^2 reached brain cortex (3%, at 1 cm deep to the skin/scalp application; Wan, Parrish et al., 1981).

Overt Naming fMRI Scan Paradigm, Pre- and Post- the LED Treatment Series: Prior to any transcranial LED treatments, structural MRI scan, and overt naming fMRI scans were obtained using the 3T Philips Scanner at the Boston University Center for Biomedical Imaging. The fMRI scans were analyzed using SPM99, and the threshold for whole brain analysis was set at p<.001, uncorrected. Our overt naming fMRI protocol uses a continuous sample, block design where the hemodynamic delay is utilized to obtain samples for "overt picture naming," during a silent period (viewing patterns), as published in Martin, Naeser et al., (2005); and Martin, Naeser, Ho et al., (2009).

Second Transcranial, LED Treatment Method for Aphasia in Stroke: *Left Hemisphere Only, LED Treatments*

The same LED device that had been used for the Bilateral LED Placement Paradigm, was used with the Left Hemisphere Only, LED treatments. During this second LED treatment method, the LED cluster heads were placed **only on the left forehead and left perisylvian language areas on the left side of the head**. A dose of 39 Joules/cm^2 (39 min 33 sec) was applied to each left hemisphere placement area. No LEDs were placed on the right hemisphere; and no LED was placed on the R and L SMAs. The same treatment schedule was used, 3x per week, for 6 weeks.

Results, fMRIs and Language Data, Pre- vs. Post- Bilateral, and Left Hemisphere Only, LED Placements

The results for the first chronic stroke patient, P1 (67 YrM), treated at 18 years post-stroke) are shown in Figs. 13 and 14.

Chronic Stroke Patient, _Bilateral_ LED Placements (_Less Effective_ t

Pre- Bilateral LED Tx.   6-1-11   **+2 Wk. Post-** 18th **Bilateral** LED Tx. 8-29-11   **+2 Mo. Pos**

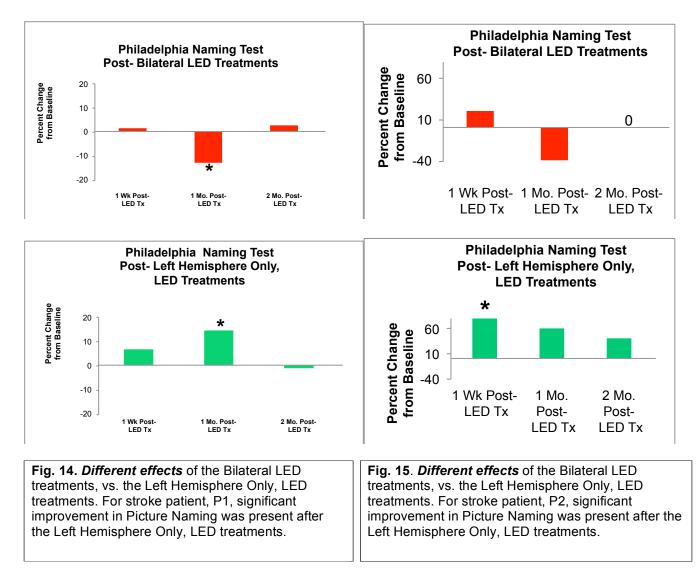## Chronic Stroke Patient, _Left Hemisphere Only_, LED Placements (_More Effe_

Pre- Left Hemisphere Only, LED Tx.
Same Scan, **as at +2 Mo.**
Post- 18th **Bilateral** LED Tx.   11-2-11

+2 Wk. Post- 18th **Left Hemisphere Only,**
LED Tx.   12-21-11
                                December 2017 Naeser - 17

+1 Mo. Pos
LED Tx.

Fig. 14. *Different effects* of the Bilateral LED treatments, vs. the Left Hemisphere Only, LED treatments. For stroke patient, P1, significant improvement in Picture Naming was present after the Left Hemisphere Only, LED treatments.

Fig. 15. *Different effects* of the Bilateral LED treatments, vs. the Left Hemisphere Only, LED treatments. For stroke patient, P2, significant improvement in Picture Naming was present after the Left Hemisphere Only, LED treatments.

Unexpectedly, after the **Bilateral LED treatments** in P1, we observed **high activation** in the **R SMA**, and in the **R frontal areas** (both areas had been treated with LED). See Fig. 13. Also, our language results showed **significantly impaired Picture Naming at +1 Mo. post- Bilateral LED treatments**, although the naming ability returned to Entry Baseline, at +2 Mo. post- Bilateral LED therapy.

In our previous fMRI aphasia research, we have observed that **high, "over-activation" in the R SMA (and other R frontal areas)** to be **compatible with poor performance on Picture Naming** (Naeser et al., 2004; Martin et al., 2005; Martin, Naeser, Ho et al., 2009). High, "over-activation" in the R frontal areas in stroke patients with nonfluent aphasia has also been observed to interfere with speech in other functional imaging studies by other investigators (Belin et al., 1996; Rosen et al., 2000; Perani et al., 2003; Postman-Caucheteux, 2010; Weiduschat et al., 2011). It is possible that R frontal "over-activation" is related to transcallosal disinhibition suggesting maladaptive plasticity and only partial, or incomplete recovery (Belin et al., 1996; Lefaucheur, 2006; Heiss and Thiel, 2006; Saur et al., 2006). The placement of LEDs on the R frontal areas and R SMA likely increased the high "over-activation" that was already present there, post-stroke. This was temporarily detrimental to speech output and Picture Naming for P1. We also observed this **same pattern of impaired Picture Naming, post- Bilateral LED Placements,** in the second stroke patient, P2 (53 YrM), treated at 12 years poststroke (Fig. 15).

Thus, our fMRI and language data suggest that **Bilateral LED placements that include R SMA and R frontal lobe, should not be used in chronic stroke patients with aphasia**, who have had left hemisphere stroke. The **LED effect** is **very focal,** and can temporarily impair naming, if the LED placements include R SMA and R frontal areas, in aphasia patients.

Both stroke patients did, however, show **significant improvement in sentence-level Auditory Comprehension, post- Bilateral LED** treatments. This is likely associated with the bilateral, increased activation in the right posterior, temporo-parietal areas, as shown in Fig. 13 top row, with stroke patient P1.

2a. 2a. iii. *Neurodegenerative Disease, Primary Progressive Aphasia:* Improved Language post-LED

Primary Progressive Aphasia is a relentless language impairment without generalized dementia (Mesulam, 1982). It is associated with progressive cortical thinning in parts of the left perisylvian language areas. One chronic PPA case, P3 (76 YrF), with the logopenic variant of PPA (lvPPA) (Gorno-Tempini et al., 2011), was treated with the **Bilateral LED treatments**. She had significant cortical thinning in her left temporo-parietal areas. She was treated during Summer 2011. She had a 2-Yr. history of PPA.

This lvPPA patient participated in overt naming fMRI scans, pre- and post- the Bilateral LED treatments. See Fig. 16. Her fMRI results are similar to those observed with the first chronic stroke patient, P1 (Fig. 13 top row). Her fMRI scan obtained at +1 Wk. Post- the Bilateral LED placements showed **increased bilateral, frontal and temporal activation**, post-LED. There was **significant improvement in Sentence-Level Auditory Comprehension** at the +2 Week Language testing, **but not Picture Naming.** There was no increased activation in either the L or R SMA (differing from chronic stroke patient, P1, Fig. 13 top row). Her follow-up fMRI scans at +1 and +2 Mo. post- LED (Fig. 16) showed the increased activation from the Bilateral LED treatments, **to wear off within 1 month**.

At +2 Mo. post- the Bilateral LED Placement Paradigm, all of her language scores remained stable compared to her Entry Baseline scores; thus there was **no overall deterioration**. Her language scores remained stable, from May 19, 2011 (pre- LED), to October 31, 2011 (+2 Mo. post- LED). The 6 weeks of LED therapy occurred in July/August. She would likely be a **good candidate for the Left Hemisphere Only**, LED treatments, as well as possibly for *long-term, Home Treatments with a LED device*.
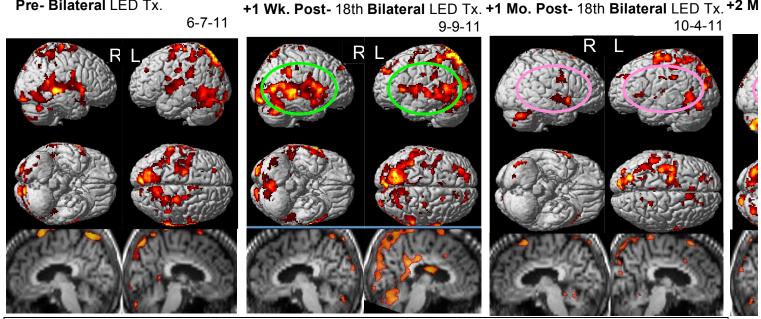

Fig. 16. P3, Neurodegenerative disease case with lvPPA. The overt naming fMRI scan obtained **at +1 Week post- the 18th Bilateral LED treatment** shows *bilateral areas of increased activation* in the R and L inferior frontal, and superior and middle, posterior temporal/inferior parietal areas (green circles). There was **significant improvement in Sentence-Level Auditory Comprehension** at the +1 Week Language testing, **but not Picture Naming.**
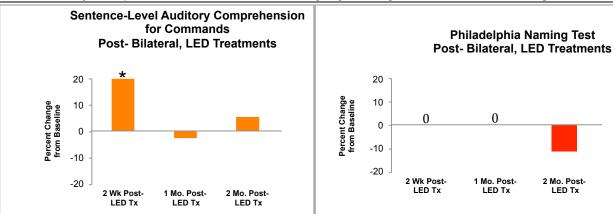
Fig. 17. Neurodegenerative Disease, P3, Primary Progressive Aphasia (PPA) similar to Alzheimer's Disease, but *Language* impairment only. **Significant improvement in sentence-level Auditory Comprehension**, post- LED. Similar to both stroke cases (P1, P2) there was impaired Picture Naming after Bilateral LED treatments. See also Figs. 13-15.

As mentioned above, P3 (neurodegenerative disease, PPA) had significant cortical thinning in the left posterior temporo-parietal area. It is of interest to note that there was **increased rCBF to her areas of atrophy,** on her fMRI scan at 1Wk., post- the 18th bilateral LED treatment, and there was significant improvement in sentence-level Auditory Comprehension at that time. These data suggest that the NIR photons are entering the brain cortex areas, even in areas where cortical thinning is already present. This suggests that additional LED treatments and LED studies are warranted with this type of neurodegenerative disease.

Her type of neurodegenerative disease, the lvPPA, has recently been studied with ^{11}C-labelled Pittsburgh Compound β PET imaging, where 12 of 13 lvPPA cases (92%) showed **positive β amyloid uptake** (Leyton et al., 2011). The effect of transcranial laser therapy (TLT) in the NIR wavelength of 808 nm (Photothera, Inc., Carlsbad, CA) was recently examined in an amyloid-β protein precursor (AβPP) transgenic **mouse model of Alzheimer's disease (AD).** After 6 Mo. of NIR TLT (3x/Wk., initiated age 3 Mo.), numbers of Aβ plaques (and amyloid load) were significantly reduced (vs. controls). **NIR TLT mitigated behavioral effects seen with advanced amyloid deposition** and **reduced the expression of inflammatory markers** in AβPP transgenic mice. The authors suggest that **NIR TLT is a potential treatment for AD** (De Taboada et al., 2011). The NIR LED treatments could also potentially help to treat neurodegenerative disease such as lvPPA, where the putative pathology is β **amyloid**.

P4 (neurodegenerative disease, with the nonfluent variant PPA, nfvPPA), is currently undergoing Left Hemisphere Only, transcranial, NIR LED treatments. She is Tau-positive from lumbar puncture, and has atyptical bilateral fronto-parietal atrophy.

Prior to any LED treatments, however, resting state, functional connectivity fMRI scans were performed pre- and post- three different doses of Joules/cm2 applied to the Left Hemisphere Only. The fMRIs were obtained, pre- and post- 1 session Sham LED treatment (0 Joules/cm2); and pre- and post- 1 session of Real LED treatment (2 Joules/cm2); and pre- and post- 1 session of Real LED treatment (13 Joules/cm2). The results shown in Fig. 18, indicate that **Sham LED had no effect**; and Real LED at a dose of only 2 J/cm2 also had no effect. Only the **Real LED** treatment **at 13 Joules/cm2** showed an increase of 1 SD, above Baseline/Entry for **strengthening the functional connections between two left frontal regions important for language, the left middle frontal gyrus and the left inferior frontal gyrus.** Both areas treated with LED.

Transcranial, Sham LED; and *Real LED Dose-Response Effect* in
Functional Connectivity Strength (resting-state fMRI) between 2 Left Hemisphere Cortical Areas for Language:
Left Middle Frontal Gyrus and Left Inferior Frontal Gyrus
Neurodegenerative Disease   72 Yr. F Primary Progressive Aphasia (PPA), Nonfluent Variant

Normal Controls - Arrows show 2 cortical areas where functional connections should be strong.

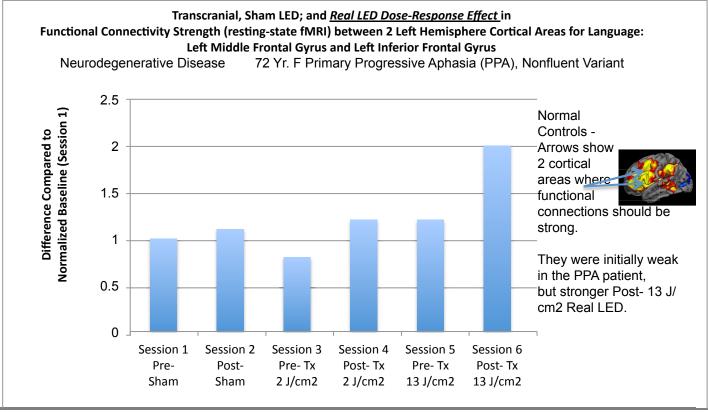They were initially weak in the PPA patient, but stronger Post- 13 J/cm2 Real LED.

Fig. 18. Neurodegenerative disease, case P4, resting state fMRI data showing functional connectivity strength between

2 left hemisphere cortical areas for language: L middle frontal gyrus and L inferior frontal gyrus. Relative to Baseline (pre-Sham), functional connectivity data show **no change post- Sham LED Treatment**, and **no change post- 2 J/cm^2 Real LED Treatment** (>2 days between paired sessions.) **Substantial increase (1 SD)** in **functional connectivity**, only **after higher dose, 13 J/cm^2 Real LED Treatment**.   Naeser, Dickerson, Ho, Martin, Treglia, Hollenbeck, Krengel, Hamblin, Baker, *in prep.*

2a. 2a. iv. Summary of Pilot Data using Transcranial, red/NIR LED to Treat 3 Patient Populations:

1. Chronic TBI, 2 published cases: Post- transcranial, red/NIR LED, there was **significant improvement in sustained attention; executive Function and verbal memory** (Naeser et al., 2011). The second case **returned to work, full-time**, after 4 months of home treatments (she had been on medical disability for 5 months.

2. Chronic TBI, 7 additional cases: Post- transcranial, red/NIR LED, there was **significant improvement in executive function (p<.01), and verbal memory (p<.05)**. Cases had cognitive dysfunction for 1 – 7 years, before entry.

3. Chronic (left-hemisphere) stroke patients with aphasia: **Post- Bilateral** transcranial, red/NIR LED treatments, there was **significant improvement in sentence-level Auditory Comprehension** (p<.05), but temporary impairment in speech (Picture Naming), each of 2 cases.

4. Chronic (left-hemisphere) stroke patients with aphasia: **Post- Left Hemisphere Only**, transcranial, red/NIR LED treatments, there was **significant improvement in Picture Naming (p<.05),** each of 2 cases.

5. fMRI scans in the chronic (left-hemisphere) stroke patient with aphasia showed that **post- Bilateral** LED treatments, there was **bilateral activation in the R and L SMA and the R and L frontal lobe areas**. This high activation in R (contralesional) frontal regions has previously been observed to interfere with speech output and Picture Naming.

6. fMRI scans in the chronic (left-hemisphere) stroke patient with aphasia showed that **post- Left Hemisphere Only** LED treatments, there was **only L SMA and L peri-lesional activation** (L sensori-motor cortex area for mouth); and there was **significant improvement in Picture Naming (p<05).**

Thus, there is a **remarkably focal effect for the specific location of LED cluster head placements on the head**, suggesting a *proof of principle*, that NIR LED light can penetrate the skin/scalp to affect brain cortex, and affect *focal* neural networks for language. The photons appear to have an effect, where they are targeted.

Bilateral LED Placements (including R and L SMA; and R and L frontal lobe areas) **significantly impaired Picture Naming**.

Left Hemisphere Only, LED Placements **significantly improved Picture Naming.**

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7. Neurodegenerative Disease, Primary Progressive Aphasia: **Post- Bilateral** transcranial, red/NIR LED treatments, there was **significant improvement in sentence-level Auditory Comprehension (p<.05)** at 2 weeks post- LED, but the effect was transient, and wore off by 1 Mo. post- LED. Post- Bilateral LED, there was no improvement in Picture Naming.

8. fMRI scans in neurodegenerative disease, Primary Progressive Aphasia (logopenic variant): **Post- Bilateral** transcranial, red/NIR LED treatments, there was **increased activation in the left posterior temporo-parietal area, where her significant cortical thinning was present.** This suggests that the NIR photons reached her primary areas of cortical thinning, and this may have been associated with her improved sentence-level Auditory Comprehension, post- Bilateral LED. Or, the increased activation in her right posterior temporo-parietal area which was also present, post- Bilateral LED could also have been associated with her improved sentence-level Auditory Comprehension.

However, the increased bilateral frontal lobe activation post- Bilateral LED, may have interfered with her Picture Naming, where there was no improvement (similar to the two stroke cases following Bilateral LED).

9. Resting state, functional connectivity fMRI scans, Neurodegenerative Disease, Primary Progressive Aphasia (nonfluent variant): Within one session of Left Hemisphere Only, red/NIR LED treatment, **Sham LED** had **no effect**, and **Real LED at 2 Joules/cm2** had **no effect**. However, **Real LED at 13 Joules/cm2 increased the functional connectivity** between two left hemisphere language areas by 1 SD (left middle frontal gyrus and left inferior frontal gyrus).

10. Taken together, the **significant improvement in executive function and verbal memory in the chronic TBI cases**; and the **significant improvement in sentence-level Auditory Comprehenson post- Bilateral LED** treatments in chronic stroke (and 1 neurodegenerative disease/PPA case) **suggest that the red/NIR photons are reaching brain cortex, to affect improvement in behavior**.

The **LEDs have a focal effect, however, ...where they are placed**. With Bilateral LED placements in chronic stroke, there is increase in bilateral activation (R and L SMA, and R and L frontal); with Left Hemisphere Only LED placement, there is increase in left hemisphere only activation (L SMA, and L peri-lesional activation).

11. Resting state, functional connectivity fMRI in neurodegenerative disease, Primary Progressive Aphasia showed a dose-response effect with LED to the Left Hemisphere Only, where there was **increased functional connectivity when 13 Joules/cm2** was applied, not 0 Joules/cm2 (Sham LED), nor 2 Joules/cm2 (Real LED).

12. The **primary, hypothesized mechanism of action** is local, **increased vasodilation** and **increased rCBF** due to **diffusion of nitric oxide outside the cell wall, following exposure of the hypoxic/ hypometabolic cells** to **red/NIR photons** from **transcranial LED.** The cytochrome C oxidase in the mitochondrial membrane is a photo-acceptor of red/NIR photons; this promotes the diffusion of nitric oxide outside the cell wall, and there is increased ATP production for improved cellular energy and function.

Our fMRI scan data obtained pre- vs. post transcranial LED therapy **support this primary, hypothesized mechanism of action,** because **increased activation and rCBF** in **specific neural networks for language** was observed post- LED. Our fMRI results are compatible with two previously published reports of increased rCBF in humans post- transcranial NIR LED – i.e., 1) with a patient in persistent vegetative state where increased rCBF was observed on SPECT scan post- a series of LED treatments (Fig. 1) (Nawashiro et al, 2011); and with severe depression cases (Fig. 2) (Schiffer et al., 2009).

> These positive effects on cognition and language behavior from our pilot studies support the **need for sham-controlled studies with transcranial LED**. This proposal will conduct a sham-controlled LED study to examine whether transcranial, red/NIR LED can be used to improve cognitive function in veterans with GWVI. There are currently, no treatments for cognitive dysfunction in veterans with GWVI.

2a. 3. Research Design and Methods

This is a blinded, randomized, sham-controlled study, originally designed as a *complete* crossover study. The study has been red-designed as a *partial* crossover study. Recruitment has been lower than expected. The new design will reduce the burden on participants for the number of visits and this is expected to improve enrollment. The new design will not alter the primary, planned statistical comparisons (Real vs. Sham LED Treatments) that are based on the 1st Series of Treatments only. The randomization and blinding remain in place.

This study investigates if scalp application of non-invasive, light-emitting diodes (LED) in red and near-infrared (NIR) wavelengths improves cognition in veterans with Gulf War Veterans' Illnesses (GWVI). Impaired cognition is one of the 3 major symptom areas of GWVI. Prior to entry, all participants will have been determined to meet the criteria of having Gulf War Veterans' Illnesses (GWVI), including cognitive dysfunction. The recruitment process and Inclusion/ Exclusion criteria are explained in detail in Section 2a. 3a. i. Participant Referral and Recruitment.

There are two groups, with about 49 participants per group; total 98; see Flowcharts, below. Participants in Group 1 receive 15 LED treatments over a 7.5 week period (2x per week, 48 hr. between visits). At +1 Week and +1 Month after the 15th Sham LED treatment (when the Post-testing has been completed), the subjects are informed that they had received the Sham LED Series of Treatments. These subjects are then offered the option of returning to receive the Real LED Series of Treatments (15 Real LED treatments over a 7.5 week period (2x per week, 48 hr. between visits), if they wish to do so. These subjects would then receive the additional payments for these extra treatment visits and Post-testing visits, for up to a total of $1,150. Participants in Group2 receive 15 Real LED treatments over a 7.5 week period (2x per week). At +1 Week and +1 Month, after the 15th Real LED treatment (when the Post-testing has been completed), the subjects are informed that they had received the Real LED Series of Treatments. The participation of these subjects is terminated at that time. They have received up to a total of $710 for their participation.

Group 1. *Sham* LED Series First; Optional *Real* LED Series Second

Time 1 Baseline Neuro-psychology Testing (1x) Actigraphy (for 1 week)	1st Intervention 15 Sham LED Tx.'s 2x/Wk, 7.5 Wks.	Times 2 and 3 Post- Testing within 1 Wk and at 1 Mo after 15th Sham LED Tx. (T_2 and T_3) Actigraphy (T_2 and T_3)	Optional 2nd Intervention 15 Real LED Tx.'s 2x/Wk, 7.5 Wks.	Times 4 and 5 Post- Testing within 1 Wk and at 1 Mo after 15th Real LED Tx. (T_4 and T_5) Actigraphy (T_4 and T_5)

Group 2. *Real* LED Series Only

Time 1	1st Intervention	Times 2 and 3		
Baseline Neuro-psychology Testing (1x) Actigraphy (for 1 week)	15 Real LED Tx.'s 2x/Wk, 7.5 Wks.	Post- Testing within 1 Wk and at 1 Mo after 15th Real LED Tx. (T_2 and T_3) Actigraphy (T_2 and T_3)		

2a. 3a. i. Participant Referral and Recruitment

Only GW veterans will participate; about 98 over the grant period. Potential participants from a Fort Devens cohort of veterans were advised in their prior informed consent procedures for previous VA-related GW studies that they may be contacted in the future about the possibility of participating in future studies. Veterans will be recruited for this study through the Co-Investigator, Maxine Krengel, Ph.D., Clinical Neuropsychologist, VA Boston Healthcare System.

Dr. Krengel is currently funded for a three-year Department of Defense grant (2011-2014) where she will re-contact the original 2,123 veterans from the Fort Devens cohort of veterans from the New England region, who returned from their 1990-91 Gulf War deployment through Ft. Devens, Massachusetts. This cohort was originally evaluated just days after their return from the Gulf War in 1991 and have been resurveyed for health symptoms and medical diagnoses multiple times in the past 21 years following their return from the war. Approximately 1,500 veterans participated in the most recent surveys of this large, population-based cohort. As part of Dr. Krengel's current resurvey of this cohort, recruitment letters will be mailed to all of the original surviving Ft. Devens cohort members. It is estimated that a minimum of 600 of these GW veterans will participate in Dr. Krengel's current DoD-funded resurvey.

These veterans will be invited by Dr. Krengel to participate in a web-based survey regarding their current health concerns and medical diagnoses. They are provided with an ID number and therefore all data are de-identified. As part of the survey, the following questionnaire is used: "Symptom Questions used to Identify Gulf War Illness by Kansas Case Definition, and Chronic Multisymptom Illness by Fukuda Case Definition." Dr. Krengel will determine from the answers to this questionnaire, whether the veterans meet the criteria from the Kansas GWI definition and the Fukuda CDC multi-symptom illness criteria for GWVI (Steele, 2000; Fukuda et al., 1998).

Additional participants may be recruited through other sources including the following.

We will submit a data request to the Department of Defense's Defense Manpower Data Center (DMDC) for the names and mailing addresses of Gulf War veterans who reside within a 100 mile radius of the Boston VA Healthcare System, JP Campus for the the purposes of study recruitment. Once we obtain the list of GW veterans who reside within a 100 mile radius, we will send them an IRB-approved recruitment letter and a permission to contact form, with a pre-addressed, stamped return envelope. These GW veterans will be invited to contact us by phone or by returning the permission to contact form if they are interested in participation and/or if they would like more information about the study. If they do not contact us or return the permission to contact form within 2 weeks, we will contact them by phone.

We would like to use CPRS to identify veterans who live locally and who meet our inclusion/exclusion criteria. We will also apply for access to the VA VINCI/CDW data, by initiating a DART data request, to obtain a list of GW veterans who reside within a 100 mile radius of the Boston VA Healthcare System, JP Campus so we can send them an IRB-approved recruitment letter and a permission to contact form, with a pre-addressed, stamped envelope. These GW veterans will be invited to contact us by phone or by returning the permission to contact form if they are interested in participation and/or if they would like more information about the study. If they do not contact us or return the permission to contact form within 2 weeks, we will contact them by phone.

After signing the ICF, at the time of neuropsychological screening, the Gulf War Veterans who are not part of the Fort Devens cohort will be given the "Symptom Questions used to Identify Gulf War Illness by Kansas Case Definition, and Chronic Multisymptom Illness by Fukuda Case Definition" questionnaire.

For the purposes of this LED study, Dr. Krengel will use the Fukuda et al., 1998 criteria for GWVI - i.e., the presence of 1 or more chronic symptoms (lasting >6 months) from at least 2 of 3 symptom categories: 1)

musculoskeletal (muscle pain, or joint pain, stiffness); 2) mood-cognition 3) fatigue. (Note, although the Fukuda et al., 1998 criteria for GWVI will be used as an Inclusion criterion for this LED study, data for the Kansas Case Definition will also be available.)

Dr. Krengel will refer veterans with GWVI who have also answered 'Yes' to the following questions: 1) Difficulty concentrating; and/or 2) Difficulty remembering recent information, to Dr. Naeser for further neuropsychological screening, and potential participation in this LED research project to improve cognition.

Additionally, recruitment, screening and data collection will occur at a second site in San Francisco, CA. This second site has been added to improve recruitment and enrollment numbers. The goal is to enroll 98 subjects for the whole study. Enrollment of 50 cases in projected for Sept. 2017 in Boston; and 50 cases is projected for March/April 2018 in SF.

Dr. Linda Chao, at SF VAMC has an active Gulf War Illness research program. In 2011 her group published a MRI paper with 64 GW Veterans who participated [NeuroToxicology 32 (2011) 814-822]. Her office will work from their most current GWI registry to recruit participants. The same protocol and processes for informed consent, enrollment, inclusion and exclusion, currently in place at the Boston VABHS (see below) will be followed. No sleep data, however, will be collected at the SF VAMC to save funds. The overall study will be overseen by Dr. Naeser.

Screening of eligibility will be conducted at the SF VAMC, using our screening criteria. Data collected at SF VAMC research site will be transferred to the Dr. Naeser's office at the VABHS, JP campus in the following manner:

A) A de-identified Data Summary Sheet for each case (will only have randomized participant ID) will be faxed to our VA FAX. It is located in one of our locked offices, and we would then place it in a locked filing cabinet in our locked office.

On the Data Summary Sheet, Age, Ed., Gender, will be provided and data timepoints will be labeled as Baseline, Testing Time 1 (T1), T2, T3, T4, etc. (no dates on this sheet).

B) We also want to be able to double-check the Data Summary Sheet with the raw data pages, just for redundancy in checking. The raw data test sheets will be mailed to us for each subject, for each testing time, so we have them for our files here. They will be kept in a locked filing cabinet, in Dr. Naeser's locked offices. This will be done through USPS Priority mail, with appropriate tracking ability. Linda Chao at the SF VA will keep copies of all the raw data sheets.The following information will be on these raw data sheets that will be xeroxed and mailed, for each subject, for each testing time: participant Id# and test dates. No name or other identifying information. Data analysis will be completed at the Boston VABHS.

2a. 3a. ia. Initial Contact and Informed Consent Process

Initial Contact. The initial contact following referral of the patient to Dr. Naeser's office by Dr. Krengel will consist of a phone call by Dr. Naeser's office to the patient. All potential participants referred by Dr. Krengel have already agreed to share their contact information regarding the possibility of participating in future GWVI studies. A description of the full study protocol, time required, and reimbursement for their time and effort will be discussed with the participants. They will be informed that whether or not they participate will have no bearing on their medical care and that, if they choose to participate, they may withdraw at any time without prejudice. After the initial contact, an appointment for obtaining signed informed consent, and further screening with neuropsychological testing at the VABHS, JP campus, will be arranged.

Informed Consent Process. If the potential participant chooses to participate in the study, an appointment will be scheduled with the PhD Study Coordinator at the VABHS, JP campus. At that time, the participant will be presented with the ICF and all questions will be answered. The participant will then be given the ICF for signature. After consent is obtained, the participant will have further screening [neuropsychological (NP) testing]. At the time of screening, the Word Reading Subtest from the Wide Range Achievement Test-4 will be used to estimate the premorbid level of cognitive functioning as a standard score for age, education, and gender (converted to SD). This score is then used for comparison to the other standardized NP test scores at screening, as follows: Participants must score at least 2 SD below their estimated premorbid cognitive level on at least 1 of these NP tests; or 1 SD below on at least 2 of these NP tests at Entry testing, for Inclusion into the study. If the participant meets the criteria for entry, based on their estimated premorbid level of cognitive functioning at NP screening on the Word Reading Subtest of the Wide Range Achievement Test (WRAT– 4), additional tests for pain, fatigue, mood and health questionnaires will be administered.

The participant will be informed that the PI will mail a letter to his/her primary care provider (PCP), informing his/her PCP about the veteran's participation. Each participant will be asked to provide the name and address of his/her PCP. A copy of that letter is provided. This letter includes a short, 2-page summary of the study. All subjects will be treated the same, and this procedure will also be followed for the GWI veterans who do have a PCP in the VA system.

Neuropsychological Screening. The neuropsychological tests that will be used in this screening battery are taken from a previously validated screening for cognitive dysfunction in Gulf War veterans (Sullivan, Krengel et al., 2003; White et al., 2001). At the time of screening, the Word Reading Subtest from the Wide Range Achievement Test-4 will be used to estimate the premorbid level of cognitive functioning as a standard score for age, education, and gender (converted to SD). This score is then used for comparison to the other standardized NP test scores at screening, as follows: Participants must score at least 2 SD below their estimated premorbid cognitive level on at least 1 of these NP tests; or 1 SD below on at least 2 of these NP tests at Entry testing, for Inclusion into the study. The following other tests are administered: 1) Trail Making Test (Reynolds, 2002); 2) COWAT - FAS Test (Spreen & Benton, 1977; Benton & Hamsher, 1989); California Verbal Learning Test II, Alternate Version (Delis et al., 2000); Stroop ("color-Word" test) (Delis, Kaplan, Kramer, 2001); and 5) Short Form McGill Pain Questionnaire (1984); 6) Overall VAS pain rating (0-10); Test of Memory Malingering (TOMM; 1996). Posttraumatic stress disorder will be assessed at screening using the PTSD Checklist –Civilian (C). The PCL-C (for civilians) scores should be >50, with PTSD symptoms exceeding clinical diagnostic thresholds for DSM-IV (i.e., 1 B-Criterion; 3 C-Criteria; 2 D-Criteria).

   After signing the ICF, at the time of neuropsychological screening, the Gulf War Veterans who are not part of the Fort Devens cohort will be given the "Symptom Questions used to Identify Gulf War Illness by Kansas Case Definition, and Chronic Multisymptom Illness by Fukuda Case Definition" questionnaire.

At screening, the Fitzpatrick Skin Type scale will be used, to determine the skin type, ranging from 1 (very fair skin) to 6 (darkest skin). Treatment dose will be adjusted when treating those participants who have the lightest (decreased) pigmentation of the skin and darkest (increased) pigmentation of the skin. See section **2a. 3b.**

If screening or neuropsychological testing cannot be completed on the same day, we will complete any remaining tests at another visit at the patient's request to accommodate their schedule.

Method of Transcranial LED Treatment
A sample data entry sheet for the Neuropsychological Screening tests and subtests is provided in Appendix 3.

2a. 3a. ib. Inclusion Criteria:
1. Must be a veteran deployed in 1990-1991 Gulf War.
2. Meets criteria for GWVI as defined by "Symptom Questions used to identify Gulf War Illness by Kansas Case Definition, and Chronic Multisymptom Illness by Fukuda Case Definition" (Steele, 2000; Fukuda et al., 1998). Participants must have the presence of 1 or more chronic symptoms (lasting >6 months) from at least 2 of 3 symptom categories from Fukuda et al., (1998): 1) musculoskeletal (muscle pain, or joint pain, stiffness); 2) mood-cognition 3) fatigue, they will be considered to have GWVI (Fukuda et al., 1998).
3. Participants must have GWVI, and have answered 'Yes' to the following questions: 1) Difficulty concentrating and/or 2) Difficulty remembering recent information, on the Symptom Questionnaire.
4. Ages 38 - 65 years.
5. Must be physically able to travel to the VA Boston Healthcare System, Jamaica Plain, or San Francisco VAMC for Neuropsychological testing and transcranial LED treatments
6. Must meet screening criteria from the Neuropsychological Screening Tests.
2a. 3a. ic. Exclusion Criteria:
1. Not meeting criteria for GWVI as defined by "Symptom Questions used to identify Gulf War Illness by Kansas Case Definition, and Chronic Multisymptom Illness by Fukuda Case Definition" (Steele, 2000; Fukuda et al., 1998).
2. Participant had GWVI, but did not answer 'Yes' to the following questions: 1) Difficulty concentrating and/or 2) Difficulty remembering recent information, on the Symptom Questionnaire.
3. Less than age 38, or greater than age 65.
4. Presence of a neurodegenerative disease such as ALS, Parkinson's, Dementia.
5. Presence of a life-threatening disease such as cancer.
6. Presence of a severe mental disorder such as schizophrenia, or severe depression.
7. Physical limitations that would prevent traveling to the VA Boston Healthcare System, Jamaica Plain, for Neuropsychological testing and transcranial LED treatments
8. Current substance abuse or active treatment
9. Did not meet screening criteria from the Neuropsychological Screening Tests.

10. Participants may not have a level of pain greater than 7/10 on VAS or 38/50 on the Short Form McGill pain questionnaires at the time of screening.

11. On the TOMM (1996), a score of less than 45 on either Part 1 or 2 would show evidence of malingering and the participant may not be included, with the following exception: If a participant fails Trial 1, but does not fail Trial 2, he/she would not be excluded if he/she also show evidence of poor learning on other NP Screening tests, such as the CVLT. If they fail Trial 2, alone, or Trial 1 and Trial 2, then this would exclude the participant from the study.

Medical records will be obtained to determine history of any surgeries; general medical history; current medications; brain CT and MRI scans with full sets of images if available, as well as Radiology reports; history of previous treatment interventions. We will request that participants let us know if there are any changes in medication type or dosages throughout their participation in this study. They will be asked weekly when they come in for treatment if there were any changes. Measurements (circumference, length from tragus of the R ear to tragus of the left ear, and length from glabella to occipital protuberance) will be taken. If the participant's head circumference is greater than 24 inches, we will utilize the Thor Helmet (for Real or Sham) to accommodate this larger head size and ensure the participant remains comfortable throughout the treatment. The treatment will be administered with appropriate timing to ensure output is equivalent. See Appendix 10. Table 1, for specifications of the Photomedex Helmet compared to the Thor Helmet.

Neuropsychological (NP) Tests: The neuropsychological tests selected as the Primary Outcome Measures for this research project measure the primary neurocognitive domains impaired in GW veterans. We have grouped them into 3 domains: 1) Attention/ Executive Function; 2) Learning and Memory; and 3) Psychomotor/Visual Spatial. Impairments measured by these tests are associated with underlying brain dysfunction involving temporal, parietal and frontal brain regions. Executive functioning is a higher-order cognitive activity that is built on more fundamental cognitive skills (i.e., attention, language, and perception). While multiple cortical regions distributed across the brain contribute to higher-order cognitive processing, the prefrontal areas of the frontal lobe (i.e., dorsolateral prefrontal cortex; anterior cingulate cortex; orbitofrontal cortex) are very involved in coordinating executive functions. These are cortical areas that are targeted (among others) with the transcranial application of red/NIR LEDs in this project. The specific NP tests are below.

Digit Span (DS): The forward and backwards span sequences of this subtest from the Wechsler Adult Intelligence Scale-IV assess attention, processing speed and working memory.

Trail Making Test (TMT): The TMT assesses the cognitive flexibility aspects of executive functioning using a visual-motor sequencing task format (i.e. connect the dots format). This study will use two variations of the TMT; one for screening (Reynolds, 2002) and the other for repeated administrations (D-KEFS Trails). TMT-Part A primarily assesses visuospatial scanning, motor sequencing skills, rote memory, integration and cognitive processing speed. TMT-Part B is more complex than Part A because it requires the examinee to connect numbers and letters in an alternating pattern (1-> A-> 2-> B-> 3-> C, etc.) as quickly as possible without errors. TMT-B has found to be a useful tool in identifying general frontal lobe dysfunctions (Reitan & Wolfson,1985; 1995). Both Part A and B are scored for time to completion and then interpreted using standard norms (Bradford, 1992; Tombaugh, 2004).

For repeated administrations of the trailmaking test, the Trails Test from the D-KEFS (trials 1-5) will used. The D-KEFS (Delis, Kaplan & Kramer, 2001) is a nationally standardized set of nine tests that measures a wide range of verbal and nonverbal executive functions. Each test is designed to be a stand-alone instrument that can be administered individually or along with other D-KEFS tests. In clinical populations, the D-KEFS can be used to assess mild brain damage in general and mild frontal-lobe involvement in particular.

Stroop Color-Word Interference Test (Stroop): The Stroop Color-Word Test is a brief, five minute test used to evaluate aspects of executive functioning, including cognitive flexibility, resistance to interference from outside stimuli, and creativity (Golden, 1978). EEG and functional neuroimaging studies of the Stroop effect have consistently revealed activation in the frontal lobe, more specifically in the anterior cingulate cortex and dorsolateral prefrontal cortex (Carter & van Veen, 2007). Four trials of the Stroop Test from the D-KFES will be administered both as a screening and an outcome measure. If the subject screens into the study based on the initial NP screening, the test is not re-administered for Baseline data; and the scores are pulled over from the NP screening, and entered as Baseline data.

California Verbal Learning Test-II (CVLT-II): The CVLT-II assesses multiple aspects of verbal learning (Delis et al., 2000). CVLT-II administration requires that a list of 16 words be presented to the examinee for memorization (4 words from 4 categories: furniture, vegetables, ways of traveling, and animals). The CVLT-II

measures both recall and recognition of the word lists over 5 different trials. It also measures delayed and cued recall. The CVLT-II has alternate forms for repeated administrations. The scores from the CVLT-II will be used for screening and as a primary outcome measure. If the subject screens into the study based on the initial NP screening, the test is not re-administered for Baseline data; and the scores are pulled over from the NP screening, and entered as Baseline data.

Continuous Performance Test-Visual AX version (CPT): The CPT measures a person's impulsivity, selective attention, plus sustained attention and effort in a cognitively demanding context. The computerized presentation of the visual CPT-AX version in this study (NES; Letz & Baker, 1988) will require the examinee to respond with a right mouse press whenever the letter stimulus on screen is an X that was preceded in the sequence by the letter "A". The left mouse button is pressed for an X that was not preceded in the sequence by the letter "A". Stimuli are presented for 200 msec each. The inter-trial interval varies across trials and may be 1.5, 2.0 or 2.5 seconds (this includes the duration of the stimulus), so the average ITI is 2.0 seconds. Approximately 20% of the stimuli are targets (A preceding X). Performances will be scored for: % correct target detections, % non-target detections, false alarms, reaction times. The CPT can be repeatedly administered.

Rey-Osterrieth Complex Figure Test (ROCF): The Rey–Osterrieth Complex Figure Test (ROCF; Osterrieth, 1944; Rey, 1941)is a widely used neuropsychological test for evaluating visuospatial-visuoconstru-ction ability and visual memory (Knight and Kaplan, 2004). It has also been used for measuring executive functions mediated by the prefrontal cortex. Examinees are asked to reproduce a complicated line drawing as a direct copy and from memory. The ROCF involves three test conditions: Copy, Immediate Recall and Delayed Recall. Each of the three productions generated is scored for accuracy and placement of 18 specific design elements according to established scoring rules. The ROCF test will be used an outcome measure.

Controlled Oral Word Association Test (COWAT)-FAS Test (Screening only): The COWAT is a brief and sensitive measure of executive function. The version used in this study appraises verbal fluency by requiring the examinee to generate as many words as possible in 60 seconds beginning with a specific letter F, A, or S. The examinee's scores is the Total Number of Words generated across the three letter categories. Both frontal and temporal lobe areas of the brain have been implicated by neuropsychological investigations as being involved in successful performances (Lezak, 1995).

2a. 3a. ii. Primary Outcome Measures, Neuropsychological Tests

The neuropsychological tests cover 3 cognitive domains: 1) Attention/Executive Function; 2) Learning and Memory; and 3) Psychomotor/Visual Spatial. A sample data entry sheet for NP tests is provided in Appendix 4.

1) Attention/Executive Function
    Digit Span Subtests (WAIS-IV; Wechsler, 2008)
    D-KEF Trails (Delis, Kaplan & Kramer, 2001)
    Stroop Test ("Color-Word" test) (Delis, Kaplan & Kramer, 2001)

2) Learning and Memory
    California Verbal Learning Test-II (CVLT-II; Delis, Kramer, Kaplan, & Ober, 2000)

3) Psychomotor/Visual Spatial
    Continuous Performance Test (Administered on laptop computer; RVisual CPT, NES)
      (Letz & Baker, 1988; Rosvold et al., 1956)
    Rey Osterrieth Complex Figure Test (ROCFT; Knight & Kaplan, 2004)

2a. 3a. iii. Secondary Outcome Measures

These additional tests measure other symptoms associated with GWVI. Sample data entry sheet, App. 4.

Pain Tests: VAS Pain Scale (0-10) (Farrar et al., 2001); Short Form McGill Pain Questionnaire (Melzack, 1984); and the West Haven-Yale Multi-dimensional Pain Inventory (WHYMPI, Kerns et al., 1985).

Fatigue: Multi-Dimensional Fatigue Inventory (Smets et al., 1995).

Sleep: Pittsburgh Sleep Quality Index (PSQI) (Buysee et al., 1989). Karolinska Sleepiness Scale (KSS). The Karolinska Sleepiness Scale will be administered each of the Neuropsychological Testing time points and each treatment day, at the end of treatment.

Actigraphy (Actiwatch): Accelerometer worn on the wrist as a watch used to provide an objective sleep measure; worn 1 week at a time, pre- treatment, 1 week and 1 month, post- Real and Sham LED treatment series. A sleep log will be filled out each week the Actiwatch is worn.

Mood: Beck Depression inventory (BDI; Beck, 2006)

Data for General Physical Health: Short Form-36V Plus (Ware et al., 2000); and the Health Symptom Checklist (HSC). The HSC is a comprehensive list of 34 frequently reported health and mental health symptoms originally adapted from Bartone et al., (1989). It asks how often in the past 30 days each of the

health symptoms was experienced. Symptoms from nine body systems are assessed (cardiac, pulmonary, dermatological, gastrointestinal, genitourinary, musculoskeletal, neurological, and psychological).

2a. 3b. Method of Transcranial LED Treatment

After all Pre-testing is complete (including the NP tests), randomization and assignment for LED treatment to Group 1 (Sham 1st, Real 2nd), or to Group 2 (Real 1st, only) will take place. A total of 49 cases are treated per Group; approximately 98 total across the study. The PhD Study Coordinator will use the free computer software program GraphPad (graphpad.com), to assign the participants (10 at a time), using a blocked randomization procedure, into the 2 groups. After 10 participants have been assigned, another set of 10 participants will be randomized into Group 1 or 2 in the same manner. This method will continue to be used, until all cases have been treated, across the study period. The study coordinator at the Boston VA is responsible for assignment of randomization at the SF VA as well.

Participants are treated 2x per week (at least 48 hours between treatments), for 7.5 weeks in each series – total of 15 LED Tx.'s per LED series. **Blinding is preserved** because neither the participant, nor the Neuro-psychologist doing the Pre- Post- testing is aware of whether the patient has received Sham or Real LED.

"Transcranial, Light-Emitting Diode (LED) Therapy to Improve Cognition in GWVI"   PI: Margaret Naeser, Ph.D.

Randomized, sham-controlled, study to examine effectiveness of transcranial LED in the near-infrared (NIR) wavelength to improve cognition (Attention, Executive Function, and Memory) in Veterans with Gulf War Veterans' Illness.

Brain Helmet made by PhotoMedex

Helmets are made up of 18 Omnilux New-U LED pods.

Omnilux New-U was FDA cleared in 2008 for reducing peri-orbital wrinkles and is sold over the counter.

Each LED pod:
Power Output 665.3 mW
Power density, 30.8 mW/cm^2
20 NIR diodes, 830nm
Emitter area: 21.6cm^2
1 J/cm^2 = 32.5 sec
26 J/cm^2 = 14 min, 5 sec.

Each session, 2 stages:
1. Midline only, 14 m, 5 s
2. R and L sides together, 14 m, 5 s
Total Treatment Time = 28 m, 10 s

Clear plastic liner, each case.

Control panel contains 5 switches. They may be used in any combination of On/Off positions.
1. On-off
2. Front only
3. Left only
4. Right only
5. Midline only

The LED Helmet manufactured by PhotoMedex, Montgomeryville, PA will be used (Fig. 19). This helmet contains 18 pods (LED cluster heads). Each pod is 4.5cm x 4.8cm. The helmet is designed with 6 pods to treat the midline of the head; 6 pods to treat the right side of the head; and 6 pods to treat the left side of the head.

The pods have been FDA-cleared as non-significant risk and a single pod is sold over-the-counter as a product labeled "OmniLux New U." This device has been FDA-cleared to apply directly to the face to reduce peri-orbital wrinkles. The FDA clearance documents are provided in Appendix 5. Each participant will be provided a clear plastic liner in the shape of the helmet, worn on the head beneath the LED Helmet. This is stored in the treatment area and labeled with the patients ID number.

The helmet liner will be re-used (after completion of all treatments for one participant) for another participant, throughout that subject's participation in the LED treatment series. The liner will be cleaned as per instructions from Infection Prevention (and the Safety Committee). See Appendix 9. CleaningofHelmetLiner6_30_16

While some warmth is produced from the LED pods in the Helmet, the warmth is dissipated by the built-in fans (…eighteen fans are used; the voltage of the fans increase from 15V to 24V in both helmets; direction of airflow is changed in 10 of the 18 fans in the Real Helmets and 4 of the 18 fans in the Sham Helmets; some warmth is applied in the Sham Helmet to make the temperature equivalent between Helmets).

Table 1 showing LED specifications for: 1) red/NIR LED cluster heads already used at VABHS (Dr. Naeser's Lab, for mTBI or stroke projects).
2) Photomedex red/NIR Helmet previously approved, January, 2013 by VABHS, Safety Subcommittee and IRB (Dr. Naeser's Lab, for GWI project)
3) Photomedex NIR ONLY Helmet, re-designed by Photomedex, June 2014, the red LEDs were removed (to reduce warmth). (for Dr. Naeser's Lab, for Gulf War Illness project)

Specific LED Parameters **Per each LED cluster head**	1) **MedX Health, Toronto**, Canada. Specifications are for each LED Cluster Head. **5-6 used simultaneously,** 2 Different Placement Sets on the head: Midline, R & L sides of the head, and on the foot.	2) **PhotoMedex Inc., Horsham**, PA. Specifications for Each LED Cluster Head (in LED Helmet). Based on Omnilux New U, pods (4 red and 20 NIR diodes per pod) embedded into the Helmet. **6, then 12 pods used simultaneously in single Tx.** 2 Different Placements Sets: a) Midline Only; then b) L and R sides only	3) **PhotoMedex Inc., Horsham**, PA. Specifications for Each LED Cluster Head/pod (in LED Helmet). **6, then 12 pods used simultaneously in single Tx.** 2 Different Placements Sets: a) Midline Only; then b) L and R sides only
LED cluster head size	2-inch diameter, 22.48 cm^2	3.9 cm x 4.8 cm, 19 cm^2	4.5 cm x 4.8 cm, 21.6 cm^2
Power output	500 mW	692.5 mW	665.3 mW
Power density	22.2 mW/cm^2	36.5 mW/cm^2	30.8 mW/cm^2
Number of red LEDs, 633 nm	9 red	4 red	No red LEDs
Number of near-infrared LEDs, 830/870 nm	52 near-infrared (870nm)	20 near-infrared (830nm)	20 near-infrared (830nm +/-5))
Continuous Wave (CW)	CW, only	CW, only	CW, only
Total Power	2.5 W (5 cluster heads on) 3.0 W (6 cluster heads on)	4.16 W (6 pods turned on) 8.3 W (12 pods turned on)	4 W (6 pods turned on) 8 W (12 pods turned on)
CW, number of sec. = 1 Joule/ cm^2	45 sec.	27.4 sec.	32.5 sec.
Treatment Dose, Joules/cm^2	13 Joules/cm^2 per Set (2 Different Sets)	26 Joules/cm^2 per Set	26 Joules/cm^2 per Set
Treatment Time per Set	9 min. 45 sec. per Set (2 Different Sets)	11 min. 52 sec. per Set	14 min. 5 sec. per Set
Total Treatment Time per Visit	19 min 30 sec. (approx. 20 19 min. 30 sec.	23 min. 44 sec.	28 min. 10 sec.

* The MedX Health LED Cluster Heads (MedX Health Console Unit Model 1100) are FDA-cleared, as non-significant risk, "...to temporarily increase blood circulation ...to temporarily decrease pain in musculoskeletal conditions." The MedX Health FDA-Clearance Documents are provided in Appendix 7.

On October 13, 2010, the Research Safety Subcommittee, VA Boston Healthcare System, approved the use of this MedX Health LED therapy system for use in Dr. Naeser's, IRB# 2492, "Transcranial LED to Improve Naming in Primary Progressive, and other Aphasias." This research project has been using this LED device since Spring, 2011. A total of 5 or 6 LED cluster heads are used on the head at the same time. The energy density doses per LED cluster head have been either 13 J/cm^2 or 39 J/cm^2 (pulsed wave, 146 Hz). There have been no negative side effects or adverse events here at the VABHS. This original Research Safety Subcommittee, VABHS, Approval document is provided in Appendix 8.

** The Photomedex LED Cluster Heads (Omnilux New-U) are FDA-cleared, as non-significant risk, "...to decrease peri-orbital wrinkles on the face." The Photomedex FDA-Clearance Documents are provided in Appendix 5.

Treatment dose adjustments for Skin Type

Treatment dose will be adjusted when treating those participants who have lighter (decreased) pigmentation or darker (increased) pigmentation of the skin. The Fitzpatrick Skin Type scale will be used, to determine the skin type, ranging from 1 (very fair skin) to 6 (darkest skin). The current treatment protocol, using 26 J/cm2 (14 min. 5 sec.) for each row of LED pods in the Photomedex Helmet, is appropriate to continue to use with skin types 2-5. Also, the current treatment protocol, using 1 J/cm2 (4 min 10 sec) on the ears, with the MedX Health LED cluster heads is appropriate to continue to use with skin types 2-5. The number of Joules/cm2, and the treatment times need to be decreased for skin type 1 and increased for skin type 6.

1.   For Part 1, and for Part 2 of the Helmet LED Treatment Protocol, for skin type 1 (very fair skin), the treatment time will be decreased by 25%. The new adjusted treatment time, per part, for type 1 will be approximately 10 min 34 sec (19.5 J/cm2).

2.   For the MedX Health LED placements on the ears, the adjusted treatment time for type 1 (where the treatment time will be decreased by 25%), the time will be 3 min 8 sec (.75 J/cm2).

3.   For Part 1, and for Part 2 of the Helmet LED Treatment Protocol, for skin type 6 (dark black), the treatment time will be increased by 25%. The new adjusted treatment time, per part, for type 6 will be approximately 17 min 36 sec (32.5 J/cm2).

4.   For the MedX Health LED placements on the ears, the adjusted treatment time for type 6 (where the treatment time will be increased by 25%), the time will be 5 min 12 sec (1.25 J/cm2).

The Sham LED helmet will look and feel the same as the Real LED helmet. The Sham LED helmet will contain the same fans activated when the switches are turned on, however no NIR photons will be emitted from the Sham LED pods. The NIR wavelength as used in the Real helmet is beyond the visible spectrum, thus the participant will not see any light during a Sham LED or a Real LED treatment.

During a Real LED treatment, a total dose of 26 J/cm^2 will be delivered from each pod (approximately 0.8 J/cm^2) is expected to reach brain cortex (Wan, Parrish, Anderson et al., 1981). The duration of the total treatment with the Sham or Real LED Helmet is about 28 minutes.

Additional LED placements
Intranasal LED Placements

At each treatment session, participants also receive a simultaneous Real or Sham Intranasal LED placement. It is hypothesized that these intranasal devices may deliver photons to the inferior temporal lobe, and hippocampus. The intranasal LED treatment procedures are painless and noninvasive. There is no risk for use of the intranasal LED device, because the LEDs are non-coherent, and thus they cannot cause any ocular damage, even if one stares directly into the red/NIR LED. **During Sham or Real LED treatment the patient wears goggles that will block the red wavelength.** A sample of the red LED is shown in the nostril in Fig. 25b.

Fig. 25b. Sample of one Intranasal, light-emitting diode (LED) device clipped in the nostril; 6.5mW. The intranasal LED uses one AA battery, and has a built-in timer for a 25 min treatment. This device does not heat up.
www.VieLight.com, Toronto, Canada

The red and near-infrared Intranasal LED devices will be used at the same time; one in each nostril, held in place by a plastic clip. The placement of the LEDs will be alternated by side (left/right) at the next session.

Each participant is provided with a red LED device and an infrared LED device that are labeled with his/her ID number, and stored in a separate plastic bag between treatments, onsite at the VABHS. It will be wiped down with an alcohol pad every day after use. The LEDs will be stored after the study in Dr. Naeser's office, with the partipant's ID label, for potential follow-up studies with each individual. The devices will not be used on another patient.

Two Intranasal LED devices (red and near-infrared) will be applied at the same time as the helmet LED treatment, Parts 1 and 2 during each LED Tx series (Sham or Real). The Intranasal placement is applied for approximately 25 min. The device has a timer that shuts itself off after 25 min. The Sham LED Intranasal devices will look and feel the same as the Real LED Intranasal devices, however, no light will be emitted.

Intranasal LED Parameters

Specifics for parameters of the VieLight, 633 red intranasal, LED device are as follows: 1) single diode; total power: 6.5mW; 633nm wavelength; power density, 7.6 mW/cm^2; energy delivered to mucosa, 11.4 J/cm^2.

Specifics for parameters of the VieLight, 810 near-infrared intranasal, LED device are as follows: 1) single diode; total power: 6.5mW (net of 50% pulsed duty cycle), pulsed at 10 Hz; 810nm wavelength; power density (net of pulsed duty cycle), 7.6 mW/cm^2; energy delivered to mucosa, 11.4 J/cm^2.

See FDA email to Dr. Naeser (7/24/13) stating that the FDA will accept the determination of risk level, as assessed by the Boston VA IRB.

MedX Health LED placements on the ears

LED cluster heads manufactured by MedX Health (Toronto) will be used for two additional transcranial LED placements on the ears. Each MedX Health Home unit contains 1 LED cluster head. Each cluster head has a 2-inch diameter, with NIR 870nm diodes. The total power is 90 mW. The transcranial LED treatment procedures are painless and noninvasive. There is no sensation of heat or pain, during LED application. There is a built-in heat-sink, so that the LED cluster heads do not become uncomfortably warm. LED therapy is considered to be a non-thermal treatment method. There is no risk for use of the transcranial LED device, because the LEDs are non-coherent, and thus they cannot cause any ocular damage, even if one stares directly into the red/NIR LED cluster head.

The MedX Health Home Unit, was **FDA-cleared as non-significant risk in 2003** and it was also **FDA-cleared for home treatment use, in 2005,** "for ...temporary increase in local blood circulation...for temporary relief of minor muscle and joint aches..." (see FDA documents)

A similar product, that contains 3 cluster heads, The MedX Health Console Units (Model 1100) is already in use at the VABHS, in Dr. Naeser's LED projects: IRB# 2492, "Transcranial LED to Improve Naming in Primary Progressive, and other Aphasias" and IRB #2703 "Transcranial LED Therapy to Improve Cognitive and Psychosocial Functioning in TBI".

During the MedX Health LED treatments, 1 J/cm2 will be applied at each LED cluster head placement. This will require 4 min 10 sec min.

There will be two MedX Health LED cluster head placements:
1) An LED cluster head is placed centered on the front of the L ear
2) An LED cluster head is placed centered on the front of the R ear

Fig 24. MedX Health Console Unit, with sample LED cluster heads to be used for the transcranial LED treatment intervention, in this research study.

Dotted white lines on MRI in **a.** and **b.,** show expected direction of NIR (870 nm) photons, into bilateral frontal lobes (prefrontal cortex and anterior cingulate gyrus areas), with forehead placements. Additional placement sites are used, see text.

Each placement is applied simultaneously with the helmet during the last 4 min of Part 2 (Left and Right) and for

The chinstrap of the LED helmet will hold the LED cluster heads into place on the front of the L and R ear. All LED cluster heads are wiped with a disinfectant, after each treatment.

All appointments are scheduled ahead, for a specific day and time of day, (usually a Mon/Weds or a Tues/Thurs schedule). It is necessary to have at least 48 hours, between treatments.

If the participant needs to miss an appointment, that appointment will be re-scheduled so that all 15 LED treatments are completed, in each of the two LED treatment series. If a subject misses 2 weeks in a row without any treatment, the subject will be given the option to start over or be withdrawn from the study.

Statistical Analyses and Power Statement *Errol Baker, PhD; Statistician, VA IRB, Consultant on this project*
Participants are likely to show different profiles for performance on individual tests on which they met entry criteria. Using all participants to examine efficacy test by test, will create a serious bias. Therefore, following the lead of the Neuropsychologists on our team (M.K. and J.K.) we will create 3 cognitive domain scores: Attention/Executive Functioning; Learning and Memory; and Psychomotor/Visual Spatial.

1) Attention/Executive Function: Digit Span Subtests (WAIS-IV; Wechsler, 2008); Trail-Making Test (Delis, Kaplan, Kramer, 2001); and Stroop Test ("Color-Word" test) (Delis, Kaplan, Kramer, 2001)
2) Learning and Memory: California Verbal Learning Test-II (CVLT-II; Delis, Kramer, Kaplan, & Ober, 2000)
3) Psychomotor/Visual Spatial; Continuous Performance Test (Administered on computer; RVisual CPT, NES3) (Letz & Baker, 1988; Rosvold et al., 1956); Rey Osterrieth Complex Figure Test (ROCF) (Knight & Kaplan, 2004)

Each of our individual tests has been standardized, and yields age/education/gender-adjusted z-scores. For each domain, we will compute the mean z-score from those tests that load for that factor (i.e., the two tests selected for Learning and Memory have a total of 9 standardized scores: the domain score will be the mean of these 9 values).

Analysis Plan:
Preliminary Analyses: Before testing any of the stated hypotheses, the frequency distributions for all study variables will be examined, and appropriate transformations will be carried out whenever violations of normality are determined. Descriptive statistics and bivariate correlations will be computed. This will be necessary in order to test for colinearity among dependent variables. The number of variables will be constrained when $r > .75$. In addition, each variable will be tested for leptokurtosis to insure that there is a sufficient range of scores. Variables found to be significantly leptokurtic will be converted into dichotomous outcomes.

Tests of Hypotheses: All participants will be evaluated at Entry (Baseline) and within one week of completing either Sham Intervention or Real Intervention The main hypothesis will be tested by two-way mixed-design analysis of variance, with a trend analysis with treatment conditions as the between-groups variable and time of testing as the repeated-measures variable. To control for the fact that the dependent variables can co-vary and for the large number of F-tests to be computed, these analyses will be carried out by Multivariate Analysis of Variance (MANOVA) utilizing PASW v.18. Subsequent to the MANOVA, a series of univariate ANOVAs will be conducted for each dependent variable. Empirical effect sizes will be computed by partial eta^2. Headsize measurements will be used as covariates.

Power Analysis:
Primary Analysis: The primary analysis is between Real Only and Sham 1st. This comparison will be between groups. It is a parallel design for this primary analysis.
Power and sample size were computed for only the primary, between-group analysis using a moderate effect size appropriate to ANOVA.
The following computer software was used: Borenstein M, Rothstein H and Cohen J. Power and Precision (2004). Englewood, NJ, Biostat. We solved for sample size in order to obtain power = .80 given the following assumptions: alpha = .05 (1-tailed); power = .80; moderate effect size. ANOVA is always a 1-tailed test.
Cohen recommends that readers of his book who consider the ES range defined as 'Large" to be too small (or too large) to meet the appropriate standards of his/her area, should define alternative operational definitions (p.79). See table created below by the PIs from the content in Cohen (1988).

Statistic	ES Index	Small	Medium	Large
Correlation	r	.10	.30	.50
Difference Between Correlations	q	.10	.30	.50
Proportion equal to .50	g	.05	.15	.25
Difference Between Proportions	h	.20	.50	.80

Chi-Square	w	.10	.30	.50
F Test on Means (ANOVA/ANCOVA) *	f	.10	.25	.40
Multiple Regression	f²	.02	.15	.35

* Please note, we used **f** as the ES Index for the power calculations submitted to the DMC.

Cohen, J. (1988). Statistical Power Analysis for the Behavioral Sciences (2nd Ed.). Lawrence Erlbaum Associates.

Secondary Analysis:
The Sham 1st shifted to Real will be a second test of efficacy of treatment, and subjects will be compared only within-subject.
In the secondary analyses the two groups will not be compared to one another.

Drop-Outs: In any longitudinal intervention study there will be participants who do not complete the protocol (missing data). The issue of missing data was addressed in a recent special report in the New England Journal of Medicine by an expert panel convened by the National Research Council (Little et al., 2012). Analyses utilizing only data from completers, even when intent-to-treat analyses have established no differences between these samples at Baseline, at best will yield an underestimation of standard errors and, at worst, can lead to biased results. They recommend using an acceptable multiple imputation (MI) procedure to "replace" missing values. Since the data are missing, in all likelihood, Not-At-Random (NAR), this will require generating a model accounting for missingness. Following the recommendations of Moon (Moon et al., 2006) we will utilize a flexible MI procedure known as MICE (multiple imputation by chained equations) on S^+ and which will utilize the outcome for imputation of missing predictor values. Completers and non-Completers will be first compared on socio-demographic variables to predict the likelihood of completion by discriminant analysis. Any variables that discriminate Completers from non-Completers will be entered into our models as weighted covariates.

Interim Analyses: For both ethical and pragmatic reasons, we have a planned interim analysis at exactly the halfway point of the study, regardless of drop-out rate. To be conservative we will employ the O'Brien-Fleming alpha spending function to establish stopping boundaries. If the MANOVA is significant at $p < .022$, efficacy will be considered to be reliably established.

Tentative Sequence or Timetable
Yr 1: Hire personnel, purchase equipment/supplies. Recruit and screen veterans. Begin treatment of 40 cases.
Yr 2: Continue to recruit and treat 40 GWVI cases. Perform interim analyses. Present papers.
Yrs 3, 4: Recruit, treat 40 GWVI cases per year. Present papers and prepare final report and manuscripts.

k. **Human Studies Section**
k.1. Risk to Subjects
k.1.a. Human Subjects Involvement and Characteristics
Only GW veterans will participate; about 98 over the grant period. All potential treatment study participants were advised in their prior informed consent procedures for previous VA-related GW studies that they may be contacted in the future about the possibility of participating in future studies. Veterans will be recruited for this study through the Co-Investigator, Maxine Krengel, Ph.D., Clinical Neuropsychologist, VA Boston Healthcare System.

Dr. Krengel is currently funded for a three-year Department of Defense grant (2011-2014) where she will re-contact the original 2,123 veterans from the Fort Devens cohort of veterans from the New England region, who returned from their 1990-91 Gulf War deployment through Ft. Devens, Massachusetts. This cohort was originally evaluated just days after their return from the Gulf War in 1991 and have been resurveyed for health symptoms and medical diagnoses multiple times in the past 21 years following their return from the war. Approximately 1,500 veterans participated in the most recent surveys of this large, population-based cohort. As part of Dr. Krengel's current resurvey of this cohort, recruitment letters will be mailed to all of the original

surviving Ft. Devens cohort members. It is estimated that a minimum of 600 of these GW veterans will participate in Dr. Krengel's current DoD-funded resurvey.

These veterans will be invited by Dr. Krengel to participate in a web-based survey regarding their current health concerns and medical diagnoses. They are provided with an ID number and therefore all data are de-identified. As part of the web-based survey, the following questionnaire is used: "Symptom Questions used to Identify Gulf War Illness by Kansas Case Definition, and Chronic Multisymptom Illness by Fukuda Case Definition." Dr. Krengel will determine from the answers to this questionnaire, whether the veterans meet the criteria from the Kansas GWI definition and the Fukuda CDC multi-symptom illness criteria for GWVI (Steele, 2000; Fukuda et al., 1998).

For the purposes of this LED study, Dr. Krengel will use the Fukuda et al., 1998 criteria for GWVI - i.e., the presence of 1 or more chronic symptoms (lasting >6 months) from at least 2 of 3 symptom categories: 1) musculoskeletal (muscle pain, or joint pain, stiffness); 2) mood-cognition 3) fatigue.

Dr. Krengel will refer veterans with GWVI who have also answered 'Yes' to the following questions: 1) Difficulty concentrating; and/or 2) Difficulty remembering recent information, to Dr. Naeser for further neuropsychological screening, and potential participation in this proposed transcranial, LED research project to improve cognition.

Additional participants may be recruited through other sources including the following.

We will submit a data request to the Department of Defense's Defense Manpower Data Center (DMDC) for the names and mailing addresses of Gulf War veterans who reside within a 100 mile radius of the Boston VA Healthcare System, JP Campus for the the purposes of study recruitment. Once we obtain the list of GW veterans who reside within a 100 mile radius, we will send them an IRB-approved recruitment letter and a permission to contact form, with a pre-addressed, stamped return envelope. These GW veterans will be invited to contact us by phone or by returning the permission to contact form if they are interested in participation and/or if they would like more information about the study. If they do not contact us or return the permission to contact form within 2 weeks, we will contact them by phone.

We would like to use CPRS to identify veterans who live locally and who meet our inclusion/exclusion criteria. We will also apply for access to the VA VINCI/CDW data, by initiating a DART data request, to obtain a list of GW veterans who reside within a 100 mile radius of the Boston VA Healthcare System, JP Campus so we can send them an IRB-approved recruitment letter and a permission to contact form, with a pre-addressed, stamped envelope. These GW veterans will be invited to contact us by phone or by returning the permission to contact form if they are interested in participation and/or if they would like more information about the study. If they do not contact us or return the permission to contact form within 2 weeks, we will contact them by phone.

Additionally, recruitment, screening and data collection will occur at a second site in San Francisco, CA. This second site has been added to improve recruitment and enrollment numbers. The goal is to enroll 98 subjects for the whole study. Enrollment of 50 cases in projected for Sept. 2017 in Boston; and 50 cases is projected for March/April 2018 in SF.

Dr. Linda Chao, at SF VAMC has an active Gulf War Illness research program. In 2011 her group published a MRI paper with 64 GW Veterans who participated [NeuroToxicology 32 (2011) 814-822]. Her office will work from their most current GWI registry to recruit participants. The same protocol and processes for informed consent, enrollment, inclusion and exclusion, currently in place at the Boston VABHS (see below) will be followed. No sleep data, however, will be collected at the SF VAMC to save funds. The overall study will be overseen by Dr. Naeser.

Screening of eligibility will be conducted at the SF VAMC, using our screening criteria. Data collected at SF VAMC research site will be transferred to the Dr. Naeser's office at the VABHS, JP campus in the following manner:

A) A de-identified Data Summary Sheet for each case (will only have randomized participant ID) will be faxed to our VA FAX. It is located in one of our locked offices, and we would then place it in a locked filing cabinet in our locked office. We have approval to receive Quest blood sample data this way.

On the Data Summary Sheet, Age, Ed., Gender, will be provided and data timepoints will be labeled as Baseline, Testing Time 1 (T1), T2, T3, T4, etc. (no dates on this sheet).

B) We also want to be able to double-check the Data Summary Sheet with the raw data pages, just for redundancy in checking. The raw data test sheets will be mailed to us for each subject, for each testing time, so we have them for our files here. They will be kept in a locked filing cabinet, in Dr. Naeser's locked offices. This will be done through USPS Priority mail, with appropriate tracking ability. The following information will be on these raw data sheets that will be xeroxed and mailed, for each subject, for each testing time: participant Id# and test dates. No name or other identifying information. Data analysis will be completed at the Boston VABHS. Linda Chao at the SF VA will keep copies of all raw data.

Neuropsychological Screening

The neuropsychological tests that will be used in this screening battery are taken from a previously validated screening for cognitive dysfunction in Gulf War veterans (Sullivan, Krengel et al., 2003; White et al., 2001). At the time of screening, the Word Reading Subtest from the Wide Range Achievement Test-4 will be used to estimate the premorbid level of cognitive functioning as a standard score for age, education, and gender (converted to SD). This score is then used for comparison to the other standardized NP test scores at screening, as follows: Participants must score at least 2 SD below their estimated premorbid cognitive level on at least 1 of these NP tests; or 1 SD below on at least 2 of these NP tests at Entry testing, for Inclusion into the study. The following tests are administered: 1) Trail Making Test (Reynolds, 2002); 2) FAS Test (Spreen & Benton, 1977; Benton & Hamsher, 1989); California Verbal Learning Test II, Alternate Version (Delis et al., 2000); and Stroop ("color-Word" test) (Delis, Kaplan, Kramer, 2001).). A sample data entry sheet for the Neuropsychological Screening tests and subtests is provided in Appendix 3. Headsize measurements (circumference, length from R tragus of the ear to L tragus of the ear, and length from glabella to occipital protuberance) will be taken. If the participant's head circumference is greater than 24 inches, we will utilize the Thor Helmet (for Real or Sham) to accommodate this larger head size and ensure the participant remains comfortable throughout the treatment. The treatment will be administered with appropriate timing to ensure output is equivalent. See Appendix 10. Table 1, for specifications of the Photomedex Helmet compared to the Thor Helmet.

If screening or neuropsychological testing cannot be completed on the same day, we will complete any remaining tests at another visit at the patient's request to accommodate their schedule.

k.1.b Sources of Materials:

All research material will be obtained specifically for research purposes, and no existing specimens, records, or data will be used. The sources of research material obtained from individually identifiable living human subjects in the form of specimens, records, or data will be obtained specifically for this research project. This includes the cognitive tests, pain questionnaires, Multi-Dimensional Fatigue Inventory; Beck Depression Inventory; Health Symptom Checklist; Short Form-36V Plus;

All subjects will be assigned a random ID number; and all records for each case will be retained in files for this research project, using that subject's ID number. All data will be kept in locked file cabinets or desks, and encrypted, password-protected computers, within locked offices. Only Dr. Naeser's co-investigators, and laboratory members associated with the study, will have access to individually identifiable private information about living human subjects.

k.1.c Potential Risks:

There is minimal risk for participating in this study. There is no risk for the paper and pencil tests for the cognitive tests, pain questionnaires, Multi-Dimensional Fatigue Inventory; Beck Depression Inventory; Health Symptom Checklist; Short Form-36V Plus. The participants may be frustrated by the difficulty of some of the neuropsychological tasks. They will receive assistance with these tasks and they can stop the session if they want to.

Actiwatch

There is no known risk of actigraphy for participants. The only potential risk may be the discomfort associated with wearing a monitor on your wrist for a continuous week. We hope to minimize this discomfort by providing comfortable bands that allow for breathability of the material while still remaining secure on the wrist.

There is a small possibility that there could be a temporary increase in symptoms, lasting up to 2 or 3 days, that are associated with Gulf War Veterans' Illnesses – these include, for example, muscle aches and pains, headache, fatigue or gastro-intestinal disorders.

There is no risk for use of the LED device, because the light-emitting diodes are non-coherent, and thus they cannot cause any ocular damage, even if one stares directly into the LED.
The participants may be sleepy after the LED treatment session for the first 2 weeks. This has been reported, especially after the first treatment. Participants will be escorted to and from the first 4 treatments (2 weeks) and advised to exercise care in going about their daily activities, immediately after the LED treatment. We will ask

the participant if they feel sleepy. The length of sleepiness may vary from case to case, and an escort will be requested, until the patient no longer exhibits excess sleepiness following an LED treatment. They will not be excluded from the study if they cannot bring an escort as requested. If they do not bring an escort and they report feeling sleepy, they will be asked to remain for an hour. If the participant prefers to leave that will be their choice.

Transcranial LED treatment.

The PhotoMedex helmet manufactured by PhotoMedex, Montgomeryville, PA will be used. This helmet contains 18 pods (LED cluster heads). Each pod is 4.5cm x 4.8cm. The helmet is designed with 6 pods to treat the midline of the head; 6 pods to treat the right side of the head; and 6 pods to treat the left side of the head. See Fig. 19 in the Research Plan. The specifications for each LED pod are as follows: Power output 665.3 mW; power density, 30.8 mW/cm2; each pod contains 24 diodes (20 NIR diodes, 830nm); 1 J/cm^2 = 32.5 sec, 26 J/cm^2 = 14 min, 5 sec; total treatment time is approximately 28 min, 10 sec.

The pods have been FDA-cleared as non-significant risk and a single pod is sold over-the-counter as a product labeled "OmniLux New U." This device has been FDA-cleared to apply directly to the face to reduce peri-orbital wrinkles. The FDA clearance documents are provided in Appendix 5.

Each participant will be provided a clear plastic liner in the shape of the helmet, worn on the head beneath the LED helmet. This is stored in the treatment area and labeled with the patients ID number.

The helmet liner will be re-used (after completion of all treatments for one participant) for another participant, throughout that subject's participation in the LED treatment series. The liner will be cleaned as per instructions from Infection Prevention (and the Safety Committee). See Appendix 9. CleaningofHelmetLiner6_30_16

Two LED helmets will be manufactured by PhotoMedex- e.g. one Real LED helmet and one Sham LED helmet. The Sham LED helmet will look and feel the same as the Real LED helmet. The Sham LED helmet will contain the same fans activated when the switches are turned on, however no NIR photons will be emitted from the Sham LED pods. The NIR wavelength is beyond the visible spectrum, thus the participant will not see any light during a Sham LED or a Real LED treatment. During a Real LED treatment, a total dose of 26 J/cm^2 will be delivered from each pod (approximately 0.8 J/cm^2 is expected to reach brain cortex (Wan, Parrish, Anderson et al., 1981). The total duration of the treatment with the Sham or Real LED helmet is about 28 minutes. The patient is treated in a recliner chair; goggles are put into place before the device is turned on.

Treatment dose adjustments for Skin Type

Treatment dose will be adjusted when treating those participants who have lighter (decreased) pigmentation or darker (increased) pigmentation of the skin. The Fitzpatrick Skin Type scale will be used, to determine the skin type, ranging from 1 (very fair skin) to 6 (darkest skin). The current treatment protocol, using 26 J/cm2 (14 min 5 sec) for each row of LED pods in the Photomedex Helmet, is appropriate to continue to use with skin types 2-5. Also, the current treatment protocol, using 1 J/cm2 (4 min 10 sec) on the ears, with the MedX Health LED cluster heads is appropriate to continue to use with skin types 2-5. The number of Joules/cm2, and the treatment times need to be decreased for skin type 1 and increased for skin type 6.
1.   For Part 1, and for Part 2 of the Helmet LED Treatment Protocol, for skin type 1 (very fair skin), the treatment time will be decreased by 25%. The new adjusted treatment time, per part, for type 1 will be approximately 10 min 34 sec (19.5 J/cm2).
2.   For the MedX Health LED placements on the ears, the adjusted treatment time for type 1 (where the treatment time will be decreased by 25%), the time will be 3 min 8 sec (.75 J/cm2).
3.   For Part 1, and for Part 2 of the Helmet LED Treatment Protocol, for skin type 6 (dark black), the treatment time will be increased by 25%. The new adjusted treatment time, per part, for type 6 will be approximately 17 min 36 sec (32.5 J/cm2).
4.   For the MedX Health LED placements on the ears, the adjusted treatment time for type 6 (where the treatment time will be increased by 25%), the time will be 5 min 12 sec (1.25 J/cm2).

Additional LED placements
Intranasal LED Placement

At each treatment session, participants also receive a simultaneous Real or Sham Intranasal LED placement. It is hypothesized that these intranasal devices may deliver photons to the inferior temporal lobe, and hippocampus. The intranasal LED treatment procedures are painless and noninvasive. There is no risk for use of the intranasal LED device, because the LEDs are non-coherent, and thus they cannot cause any ocular

damage, even if one stares directly into the red/NIR LED. **During Sham or Real LED treatment the patient wears goggles that will block the red wavelength.**

The red and near-infrared Intranasal LED devices will be used at the same time; one in each nostril, held in place by a plastic clip. The placement of the LEDs will be alternated by side (left/right) at the next session.

Each participant is provided with a red LED device and an infrared LED device that are labeled with his/her ID number, and stored in a separate plastic bag between treatments, onsite at the VABHS. It will be wiped down with an alcohol pad every day after use. The LEDs will be stored after the study in Dr. Naeser's office, with the partipant's ID label, for potential follow-up studies with each individual. The devices will not be used on another patient.

Two Intranasal LED devices (red and near-infrared) will be applied at the same time as the helmet LED treatment, Parts 1 and 2 during each LED Tx series (Sham or Real). The Intranasal placement is applied for approximately 25 min. The device has a timer that shuts itself off after 25 min. The Sham LED Intranasal devices will look and feel the same as the Real LED Intranasal devices, however, no light will be emitted.

Intranasal LED Parameters

Specifics for parameters of the VieLight, 633 red intranasal, LED device are as follows: 1) single diode; total power: 6.5mW; 633nm wavelength; power density, 7.6 mW/cm^2; energy delivered to mucosa, 11.4 J/cm^2.

Specifics for parameters of the VieLight, 810 near-infrared intranasal, LED device are as follows: 1) single diode; total power: 6.5mW (net of 50% pulsed duty cycle), pulsed at 10 Hz; 810nm wavelength; power density (net of pulsed duty cycle), 7.6 mW/cm^2; energy delivered to mucosa, 11.4 J/cm^2.

See FDA email to Dr. Naeser (7/24/13) stating that the FDA will accept the determination of risk level, as assessed by the Boston VA IRB.

MedX LED placements

LED cluster heads manufactured by MedX Health (Toronto) will be used for two additional transcranial LED placements on the ears. Each MedX Health Home unit contains 1 LED cluster head. Each cluster head has a 2-inch diameter, with NIR 870nm diodes. The total power is 90 mW. The transcranial LED treatment procedures are painless and noninvasive. There is no sensation of heat or pain, during LED application. There is a built-in heat-sink, so that the LED cluster heads do not become uncomfortably warm. LED therapy is considered to be a non-thermal treatment method. There is no risk for use of the transcranial LED device, because the LEDs are non-coherent, and thus they cannot cause any ocular damage, even if one stares directly into the red/NIR LED cluster head.

The MedX Health Home Unit, was **FDA-cleared as non-significant risk in 2003** and it was also **FDA-cleared for home treatment use, in 2005,** "for ...temporary increase in local blood circulation...for temporary relief of minor muscle and joint aches..." (see FDA documents)

A similar product, that contains 3 cluster heads, The MedX Health Console Units (Model 1100) is already in use at the VABHS, in Dr. Naeser's LED projects: IRB# 2492, "Transcranial LED to Improve Naming in Primary Progressive, and other Aphasias" and IRB #2703 "Transcranial LED Therapy to Improve Cognitive and Psychosocial Functioning in TBI".

During the MedX Health LED treatments, 1 J/cm2 will be applied at each LED cluster head placement on each ear. This will require 4 min 10 sec.

There will be two MedX Health LED cluster head placements:

1) An LED cluster head is placed centered on the front of the L ear

2) An LED cluster head is placed centered on the front of the R ear

Each placement is applied simultaneously with the helmet during the last 4 min of Part 1 (Midline). The chinstrap of the LED helmet will hold the LED cluster heads into place on the front of the L and R ear. All LED cluster heads are wiped with a disinfectant, after each treatment.

All appointments are scheduled ahead, for a specific day and time of day, (usually a Mon/Weds or a Tues/Thurs schedule). It is necessary to have at least 48 hours, between treatments.
If the participant needs to miss an appointment, that appointment will be re-scheduledso that all 15 LED Treatments are completed, in each of the two LED Treatment Series. If a subject misses 2 weeks in a row without any treatment, the subject given the option to start over, or be withdrawn from the study.

k.1.c.1. Therapeutic Risk:

There is no therapeutic risk involved with this project.

There is potential for sleepiness immediately following the first 4 LED treatments. The length of sleepiness may vary from case to case, and if possible an escort will be required, until the patient no longer exhibits excess sleepiness following an LED treatment. We will ask the participant if they feel sleepy. The length of sleepiness may vary from case to case, and an escort will be requested, until the patient no longer exhibits excess sleepiness following an LED treatment. They will not be excluded from the study if they cannot bring an escort as requested. If they do not bring an escort and they report feeling sleepy, they will be asked to remain for an hour. If the participant prefers to leave that will be their choice.

k.2 Adequacy of Protection from Risk

k.2.a Recruitment and Informed Consent

Only GW veterans will participate; 98 over the grant period. All potential treatment study participants were advised in their prior informed consent procedures for previous VA-related GW studies that they may be contacted in the future about the possibility of participating in future studies. Veterans will be recruited for this study through the Co-Investigator, Maxine Krengel, Ph.D., Clinical Neuropsychologist, VA Boston Healthcare System.

Dr. Krengel is currently funded for a three-year Department of Defense grant (2011-2014) where she will re-contact the original 2,123 veterans from the Fort Devens cohort of veterans from the New England region, who returned from their 1990-91 Gulf War deployment through Ft. Devens, Massachusetts. This cohort was originally evaluated just days after their return from the Gulf War in 1991 and have been resurveyed for health symptoms and medical diagnoses multiple times in the past 21 years following their return from the war. Approximately 1,500 veterans participated in the most recent surveys of this large, population-based cohort. As part of Dr. Krengel's current resurvey of this cohort, recruitment letters will be mailed to all of the original surviving Ft. Devens cohort members. It is estimated that a minimum of 600 of these GW veterans will participate in Dr. Krengel's current DoD-funded resurvey.

These veterans will be invited by Dr. Krengel to participate in a web-based survey regarding their current health concerns and medical diagnoses. They are provided with an ID number and therefore all data are de-identified. As part of the web-based survey, the following questionnaire is used: "Symptom Questions used to Identify Gulf War Illness by Kansas Case Definition, and Chronic Multisymptom Illness by Fukuda Case Definition." Dr. Krengel will determine from the answers to this questionnaire, whether the veterans meet the criteria from the Kansas GWI definition and the Fukuda CDC multi-symptom illness criteria for GWVI (Steele, 2000; Fukuda et al., 1998).

For the purposes of this LED study, Dr. Krengel will use the Fukuda et al., 1998 criteria for GWVI - i.e., the presence of 1 or more chronic symptoms (lasting >6 months) from at least 2 of 3 symptom categories: 1) musculoskeletal (muscle pain, or joint pain, stiffness); 2) mood-cognition 3) fatigue.

Dr. Krengel will refer veterans with GWVI who have also answered 'Yes' to the following questions: 1) Difficulty concentrating; and/or 2) Difficulty remembering recent information, to Dr. Naeser for further neuropsychological screening, and potential participation in this proposed transcranial, LED research project to improve cognition.
Additionally, recruitment, screening and data collection will occur at a second site in San Francisco, CA. This second site has been added to improve recruitment and enrollment numbers. The goal is to enroll 98 subjects for the whole study. Enrollment of 50 cases in projected for Sept. 2017 in Boston; and 50 cases is projected for March/April 2018 in SF.
Dr. Linda Chao, at SF VAMC has an active Gulf War Illness research program. In 2011 her group published a MRI paper with 64 GW Veterans who participated [NeuroToxicology 32 (2011) 814-822]. Her office will work from their most current GWI registry to recruit participants. The same protocol and processes for informed consent, enrollment, inclusion and exclusion, currently in place at the Boston VABHS (see below) will be followed. No sleep data, however, will be collected at the SF VAMC to save funds. The overall study will be overseen by Dr. Naeser.
Screening of eligibility will be conducted at the SF VAMC, using our screening criteria. Data collected at SF VAMC

research site will be transferred to the Dr. Naeser's office at the VABHS, JP campus in the following manner:

A) A de-identified Data Summary Sheet for each case (will only have randomized participant ID) will be faxed to our VA FAX. It is located in one of our locked offices, and we would then place it in a locked filing cabinet in our locked office. We have approval to receive Quest blood sample data this way.

On the Data Summary Sheet, Age, Ed., Gender, will be provided and data timepoints will be labeled as Baseline, Testing Time 1 (T1), T2, T3, T4, etc. (no dates on this sheet).

B) We also want to be able to double-check the Data Summary Sheet with the raw data pages, just for redundancy in checking. The raw data test sheets will be mailed to us for each subject, for each testing time, so we have them for our files here. They will be kept in a locked filing cabinet, in Dr. Naeser's locked offices. This will be done through USPS Priority mail, with appropriate tracking ability. The following information will be on these raw data sheets that will be xeroxed and mailed, for each subject, for each testing time: participant Id# and test dates. No name or other identifying information. Data analysis will be completed at the Boston VABHS. Linda Chao at the SF VA will keep copies of all raw data sheets.

Recruitment Methodology and description of informed consent process
Participants will be recruited until 98 participants have completed the treatment trial.

Initial Contact. The initial contact following referral of the patient to Dr. Naeser's office by Dr. Krengel will consist of a phone call by Dr. Naeser's office to the patient. All potential participants referred by Dr. Krengel have already agreed to share their contact information regarding the possibility of participating in future GWVI studies. A description of the full study protocol, time required, and reimbursement for their time and effort will be discussed with the participants. They will be informed that whether or not they participate will have no bearing on their medical care and that, if they choose to participate, they may withdraw at any time without prejudice. After the initial contact, an appointment for obtaining signed informed consent, and further screening with neuropsychological testing at the VABHS, JP campus, will be arranged.

Informed Consent Process. If the potential participant chooses to participate in the study, an appointment will be scheduled with the PhD Study Coordinator at the VABHS, JP campus. At that time, the participant will be presented with the ICF and all questions will be answered. The participant will then be given the ICF for signature. After consent is obtained, the participant will have further screening [neuropsychological (NP) testing]. At the time of screening, the Word Reading Subtest from the Wide Range Achievement Test-4 will be used to estimate the premorbid level of cognitive functioning as a standard score for age, education, and gender (converted to SD). This score is then used for comparison to the other standardized NP test scores at screening, as follows: Participants must score at least 2 SD below their estimated premorbid cognitive level on at least 1 of these NP tests; or 1 SD below on at least 2 of these NP tests at Entry testing, for Inclusion into the study. If the participant meets the criteria for entry, additional tests for pain, fatigue, mood and health questionnaires will be administered.

Neuropsychological Screening. The neuropsychological tests that will be used in this screening battery are taken from a previously validated screening for cognitive dysfunction in Gulf War veterans (Sullivan, Krengel et al., 2003; White et al., 2001). At the time of screening, the Word Reading Subtest from the Wide Range Achievement Test-4 will be used to estimate the premorbid level of cognitive functioning as a standard score for age, education, and gender (converted to SD). This score is then used for comparison to the other standardized NP test scores at screening, as follows: Participants must score at least 2 SD below their estimated premorbid cognitive level on at least 1 of these NP tests; or 1 SD below on at least 2 of these NP tests at Entry testing, for Inclusion into the study. The following tests are administered: 1) Trail Making Test (Reynolds, 2002); 2) FAS Test (Spreen & Benton, 1977; Benton & Hamsher, 1989); California Verbal Learning Test II, Alternate Version (Delis et al., 2000); and Stroop ("color-Word" test) (Delis, Kaplan, Kramer, 2001). A sample data entry sheet for the Neuropsychological Screening tests and subtests is provided in Appendix 3.

k.2.b Protection Against Risk
The protections against the minimal risks involved in this study were explained in detail, above. These include advising the patient to come for the LED treatments with an escort, for at least the first 4 LED treatments or remain for an extra hour because the patient may have excess sleepiness after treatment.

The risks to confidentiality for the data in the study will be secured by the assignment of a subject ID number, for each participant, for the files kept at the VABHS, JP campus. These files will be kept in a locked filing cabinet, in the locked office, of the Study Coordinator at the VABHS, JP campus. All data entered into the computer will be de-identified, using only the patient's subject ID number.

Most of the GWVI cases who will be participating in this study, do not have their medical care managed at the VABHS, JP campus. If there is a medical emergency during the LED treatment, emergency services would be called immediately, in the event of a serious emergency. There have been no negative side effects or adverse events with any of the LED treatment methods proposed for use in this study.

Adverse events, not previously identified, will be documented and reported to the VA Boston Healthcare System, IRB in a timely manner.

Each patient will be provided with his/her own clear plastic liner for the helmet. This is labeled with their ID and stored in a separate plastic bag between treatments. The bag is stored onsite at VABHS, JP campus.

The helmet liner will be re-used (after completion of all treatments for one participant) for another participant, throughout that subject's participation in the LED treatment series. The liner will be cleaned as per instructions from Infection Prevention (and the Safety Committee). See Appendix 9. CleaningofHelmetLiner6_30_16

k.3. Potential benefits of the Proposed research to the Subjects and Others

There are no known benefits of this research for subjects with GWVI, who wish to participate. Recent case report studies have observed that when red/NIR laser or LED was applied transcranially (through forehead and scalp), there was reduced severity in acute and chronic stroke patients; reduced severity in major depression cases; and significantly improved cognition in chronic, mild traumatic brain injury cases. Given that there have been no reports of negative side effects, or adverse events, in cases treated with transcranial, low-level laser, or light-emitting diode therapies, the possible benefits for the GWVI cases who have cognitive dysfunction outweigh any potential risks (the LEDs are FDA-cleared and nonsignficant risk). Results from this LED study are anticipated to benefit the GW veterans, as well as others.

k.4. Importance of knowledge to be gained

There are currently no known treatments for the cognitive dysfunction in veterans with GWVI. This pilot, clinical trial treatment study investigates a new light-emitting diode (LED) therapy to improve cognition in veterans with GWI. Approximately 175,000 – 209,000 of the 697,000 Gulf War deployed veterans report persistent, multi-symptom illnesses. These symptoms accord closely with those of **mitochondrial dysfunction**. LED therapies in the red and near infrared (NIR) wavelengths **improve mitochondrial function** by increasing adenosine tri-phosphate (ATP) production for energy metabolism, and improving cellular respiration. Treating a mechanistic-based cause (mitochondrial dysfunction), as well as symptoms, in the nearly 175,000 – 209,000 ill Gulf War veterans remains a top priority for government agencies and advisory panels (RAC 2008). The results from this study may have application to improving cognition in other central nervous system disorders, such as traumatic brain injury and stroke.

k.5. Resources

k.5.a. Research Space

Two additional treatment rooms will be necessary located near Dr. Naeser's offices, on the 12th Floor, D-Wing. A request to the Space Committee has been made; Rooms D12-97B and D12-97D are possibilities.

This proposed project requires the use of two additional treatment rooms, so that two patients can be treated at the same time, in separate rooms. Each treatment room would have 1 medical recliner chair, and 1 utility cart. One treatment room will have the Photomedex, Real LED Helmet, and one will have the Photomedex, Sham LED Helmet. The Photomedex, LED Helmet will be stored on the utility cart. Each utility cart has shelves, and wheels, so that it can be placed in close proximity to the participant in the medical recliner chair, for each transcranial LED treatment.

k.5.b. Other Research Resources

Location: VA Boston Healthcare System, Jamaica Plain campus:

Margaret Naeser, Ph.D., PD/PI, has an office located at the VA Boston Healthcare System, Jamaica Plain Campus, 12th Floor, D-Wing (12D-87).

Maxine Krengel, Ph.D., Co-investigator (Health Science Specialist, Clinical Neuropsychologist), would need temporary office space, one day a week.

Jeffrey Knight, Ph.D., Co-investigator, has an office located at the VA Boston Healthcare System, Jamaica Plain Campus, 12th Floor, B-Wing, in the National Center for PTSD.

Paula Martin, Study Coordinator, has office space at this time, within Dr. Naeser's offices.

Michael Ho, Ph.D., Co-investigator, has office space at this time, within Dr. Naeser's offices.

There is space for a research assistant, within Dr. Naeser's offices.

Additional office furniture (locked desk, locked filing cabinet), for placement of the laptop computer (encrypted and dedicated to this research project), and the patient records and data files will be necessary, however, to have in each of the two additional treatment rooms.

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