INVESTIGATION OF PREFRONTAL COGNITION RESPONSES TO TRANSCRANIAL LASER STIMULATION IN PTSD USING FUNCTIONAL NEAR INFRARED SPECTROSCOPY

by

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Abstract

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In recent years low level laser therapy (LLLT) has been of great research interest because of its therapeutic applications in psychiatry, neurology and ophthalmology. In-vivo transcranial delivery of LLLT has shown to induce beneficial metabolic effects and increase in cognitive functions and hence has been found to be useful in treating neurodegenerative disorders. The aim of this study was to evaluate effects of LLLT on prefrontal cognitive functions, namely attention and short term memory, over six sessions on veterans with Post-Traumatic Stress Disorder (PTSD), using near infrared spectroscopy (fNIRS). In this study the subjects were instructed to perform two neurocognitive tasks, namely Psychomotor Vigilance Task (PVT) and Delayed Match-to-Sample task (DMS). Sustained attention of each individual was evaluated by PVT and DMS was used to evaluate short-term memory. fNIRS was used to measure to hemodynamic responses occurring while performing the tasks. We first measured the behavioral and hemodynamic responses of healthy control subjects while performing the two tasks without administering laser stimulation. Each veteran with PTSD was given six treatment session. In each session they were instructed to perform the two tasks before and after LLLT.

We evaluated the effect of LLLT on veterans over six treatment sessions. We also evaluated the pre and post treatment effects over six sessions. We then compared the results of controls with that of the veterans before they underwent the first treatment session i.e. pre-LLLT 1, in order to see if there is any difference between their performances. We also compared the results of controls with that of veterans after receiving the last treatment i.e. post-LLLT 6, in order to see if veterans had an improved cognitive function after receiving all six treatments.

The results indicated that LLLT had a beneficial effect on PTSD subjects for the DMS task. However, LLLT did not show any significant effect on the PTSD subjects for the PVT task.

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Chapter 1

Introduction

1.1 Functional Near Infrared Spectroscopy

Functional Near-Infrared Spectroscopy (fNIRS) is a non-invasive, safe, portable and low-cost method of directly or indirectly monitoring brain activity. It uses the NIRS principle to map brain activity. Below 650nm hemoglobin is a strong absorber of light whereas light above 950nm is heavily absorbed by water. However, between 650-950nm the combined absorption of light from hemoglobin and water is minimal and is known as the optical window or therapeutic window for optical imaging. In this wavelength range scattering is most dominant interaction of light photons in the human tissue than absorption. Light diffuses through the tissue as a result of which light can penetrate through several centimeters of thickness. The absorption spectra of HbO (oxy-Hemoglobin), Hb (deoxy-Hemoglobin) and water in the optical window is as shown in Figure 1.

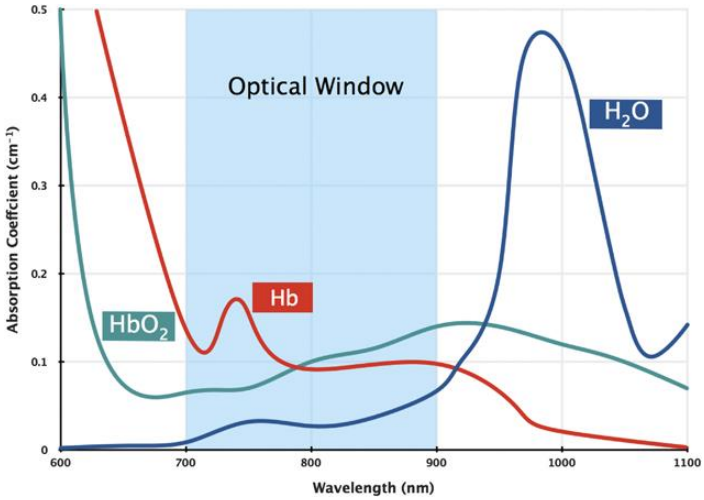


Figure 1: Absorption Spectra of HbO, Hb and H_2O in the optical window [1]

From the figure we can see that the HbO and Hb spectra are different and they can be separated by a few wavelengths. Changes in HbO and Hb concentrations can be measured in the optical window.

1.1.1 Light tissue interactions

Light propagation inside the tissue can be described in terms of photon flux. Photons, while travelling inside the tissue, can undergo absorption, scattering (elastic or inelastic) and fluorescence. When photon is absorbed, the energy of the photon is either lost as heat or given off as luminescence. Photon is either scattered by stationary tissues or by moving scatterers such as blood cells [2]. Most of the tissues are characterized by strong optical scattering and are referred to as scattering or turbid media [3].

a) Absorption:

The main absorbers of NIR light in tissues are HbO, Hb and water. However, there are other absorbers of NIR light such as myoglobin, lipids, cytochrome oxidase, melanin, bilirubin and etc. Tissue's absorption properties are described by the absorption coefficient (μ_a). Absorption coefficient gives the probability of photon absorption in a medium per unit path length and is the inverse of average photon path length before the photon gets absorbed. Typical value of μ_a in biological tissue is 0.1 cm^{-1} [3]. For a medium containing many absorbers, the absorption coefficient is given as: -

$$\mu_a = N_a \sigma_a \quad (1.1)$$

where N_a= Number of absorbers and σ_a=Absorption cross-sectional area of an absorber.

As light propagates through absorbing-only medium, it undergoes attenuation which is given as: -

$$\frac{dI}{I} = \mu_a \, dx \tag{1.2}$$

where I is the light intensity and x denotes distance along the light propagation direction. This equation represents the percentage of light being absorbed in interval (x, x+dx) and is proportional to the product of μ_a and dx [3]. Integrating equation 1.2 gives:

$$I(x) = I_o \exp(-\mu_a x) \tag{1.3}$$

The above equation is known as the Beer Lambert Law (BLL). Where I_o is the light intensity at x=0. The negative sign indicates that I decrease as x increases.

The absorption coefficient of biological tissues is wavelength dependent and hence we can calculate the coefficient by estimating the chromophore concentrations at particular wavelengths by the below equation [3, 4]: -

$$\mu_a(\lambda) = 2.3 * [\varepsilon_1(\lambda) \cdot C_1 + \varepsilon_2(\lambda) \cdot C_2 \ldots\ldots\ldots\ldots \cdot C_n] \tag{1.4}$$

where $\varepsilon_i(\lambda)$ is the absorption extinction co-efficient for concentration C_i for wavelength λ. If the absorption coefficients are known at specific wavelengths we can calculate the concentrations of the chromophores by solving simultaneous equations.

b) Scattering:

In biological tissues photons are scattered by cells and cell organelles. In tissues, light is mainly scattered in forward direction. Scattering in tissues is described by scattering coefficient (μ_s) which is defined as, probability of photon scattering in a medium per unit path length and is the inverse of average photon path length between successive scattering events. The scattering coefficient for a medium containing many scatterers is given by:

$$\mu_s = N_s \, \sigma_s \tag{1.5}$$

where N_s= Number of scatterers and σ_s= Scattering cross-sectional area of a scatterer.

When a photon undergoes multiple scattering, we consider the reduced scattering coefficient μ_s' which is defined as, inverse of the average distance over which the direction of propagation of a photon is randomized and is given as:

$$\mu_s' = \mu_s (1-g) \quad (1.6)$$

where g=anisotropy factor, which is the cosine of scattering angle and is typically 0.8-0.9 for biological tissues [3].

As light propagates through the tissue, it diffuses in all directions and has a banana shaped trajectory.

1.1.2 Modified Beer-Lambert's Law

For media, where scattering is negligible and absorption dominates light attenuation, the transmitted intensity is given by the Beer Lambert Law (BLL). However, for highly scattering media, for example biological tissues, attenuation of light is due to a combination of both absorption and scattering [5]. As the photons are scattered in the media, they follow a random walk and the average path length of the photon is greater than the straight line distance between source and detector and hence have a banana shaped profile. This propagation of NIR light can be quantified by using the Modified Beer-Lambert Law (MBLL). MBLL takes scattering into account in a turbid medium by making use of mean path length as an estimator of the actual photon path length. MBLL is as given below, which determines the optical density: -

$$OD = \log\frac{I_0}{I} = \epsilon cL = \frac{\mu_a L}{2.3} \quad (1.7)$$

where, OD is the optical density, I_o is the light intensity before the change of concentration, I is the light intensity after the change of concentration, c is the concentration of the absorbing species, ϵ is the extinction coefficient of the absorbing species, and L is the path length through the tissue.

The true optical distance also known as differential path length, which is L in this case, is given as: -

$$L=d.DPF \tag{1.8}$$

where d=geometric distance i.e. the distance between the source and the detector, DPF=Differential Path Length Factor.

MBLL gives a relationship between change in optical density and change in absorption coefficient and is given as [6]: -

$$\Delta OD = \frac{\Delta \mu a * L}{2.3} \tag{1.9}$$

MBLL can be used to monitor chromophore (hemoglobin) concentration changes in the brain and can be given as:

$$\Delta OD = \epsilon \Delta c L \tag{1.10}$$

1.1.3 FNIRS Instrumentation

There are 3 main types of NIR light spectrometers namely: continuous wave NIRS, time domain NIRS and frequency domain NIRS. In continuous wave mode, the tissue is illuminated at constant amplitude and frequency, and only the light attenuation through the tissue is measured. It is the most common form of NIRS and can be used to measure absolute changes in chromophore (oxy and deoxy-hemoglobin) concentration with the help of mBLL. The second technique is the time domain NIRS in which, tissue is illuminated with short NIR pulses and the photons undergo scattering and the photons transmitted are counted and sorted based on their time of arrival. In frequency domain technique, head is shined with amplitude modulated NIR light and the changes in amplitude and phase of the back scattered light is measured, which would provide information about the changes in the concentration of hemoglobin. Either of the three types of spectrometer is selected based on the type of information one needs to collect.

A fiber optic laser and/or light emitting diode is used as the light source in FNIRS device and fiber optic detectors. Fiber optics has an advantage in that it can fit to any head posture and position. By having a source detector distance of around 3 cm we can have adequate penetration of NIR light [4, 7]. However, the source detector distance selection depends on the wavelength, intensity, the brain area being measured and the age of the subject.

1.2 Low-Level Laser Therapy (LLLT)

Low level light therapy (LLLT) uses near-infrared light energy for therapeutic purposes. LLLT uses light energy to modify the biological functions of cells and is hence known as photobiomodulation. The advantage of using LLLT is it has no harmful effects on the cells as the power used is low compared to other techniques. The main principle behind LLLT is that certain molecules in biological system absorb light photons and are excited to higher energy levels, modifying the cellular function as a whole, which activates certain pathways.

LLLT can be defined as the use of directional low-power and high-fluence monochromatic or quasimonochromatic light from lasers or light-emitting diodes (LEDs) in the red to near-infrared wavelengths (λ = 600–1100 nm) to modulate a biological function or induce a therapeutic effect in a non-destructive and non-thermal manner. [8, 9]

1.2.1 Light Source

Main sources of LLLT are LED arrays and lasers. Laser is a coherent light source which produces light of single wavelength. It has advantages like high penetration depths and constant beam width which will concentrate the energy over a specific area. The beam width can be adjusted by coupling it with fiber optics. LED source on the other hand is a non-coherent source and produces light in a narrow range of wavelength. While lasers are capable of heat production that can induce tissue damage, LEDs generate negligible amounts of heat, thus reducing the risk of thermal injury [10].

There is no risk of thermal injury at low irradiances if the laser output power is chosen correctly. The parameters to be considered while performing LLLT is as shown in the table 1.

Table 1: Parameters of LLLT [9]

Parameter	Unit	Explanation
Wavelength	nm (nanometers)	Wavelength (λ) is the distance between wave peaks. Light is a form of energy with wave behavior. Photoacceptors exhibit different sensitivities to different wavelengths. The most effective LLLT wavelength range is 600–1100 nm. Light visible to the human eye is 400–700 nm. The higher the wavelength the lower the energy.
Energy	J (joules)	Energy (E) is the frequency (v) of radiation by Planck's constant (h) of 6.626×10^{-34} J sec (E = hv). Energy of a photon depends on the frequency of radiation (Ephoton = hv). A photon is a particle of electromagnetic radiation with zero mass and a quantum of energy (minimum E gained or lost by atom). Energy (J) = Power (W) × Time (seconds).
Power	W (Watts)	Amount of energy (J) transferred or flowing per unit of time (W = J/seconds).
Irradiance	W/cm²	Power (W) per surface area (cm²). Also called power density or light "intensity". Irradiance = Power (W) / Area (cm²).
Radiant exposure	J/cm²	Energy (J) per surface area (cm²). Equivalent to power density per unit of time (seconds). Also called fluence, energy density, or light "dose." Thus, "dose" can be easily varied by changes in exposure time. However, at the same energy density (J/cm²) variations in either irradiance (W/cm²) or time may cause different LLLT effects on tissues.
Exposure time	Seconds	Time during which the target tissue is exposed to light.
Wave type	Continuous versus pulsed	Continuous waves may be advantageous for transcranial applications. Pulse waves may decrease thermal effects. Pulse Average Power = Peak Power (W) × Pulse Width (seconds) × Pulse Frequency (Hz).
Fraction protocol	Number of fractions	Total dose can be divided in treatment sessions or fractions of specific duration and separated by specific intervals of time (eg, minutes, hours, days).
Aperture	Area of the light beam	Can be parallel, convergent, or divergent. Aperture may influence efficiency and tissue penetration.
Delivery mode	Distance of the beam source to the target tissue	Types: shallow (or noncontact), contact, and deep. Shallow is preferable when larger areas need to be exposed, but offers lower tissue penetration for light-emitting diodes. Deep delivery implicates pressure of the beam source on the target tissue.

1.2.2 Biological target

Basically, there are two types of light absorbing molecules in living systems, namely specialized cells and non-specialized. The specialized molecules known as photoreceptors significantly convert energy and include molecules like chlorophyll, rods and cones. The non-specialized molecules also absorb light but are not involved in processing light and are known as photoacceptors. Unlike the photoreceptors, which are confined to specialized cells, photoacceptors

are found in abundance and are mainly involved in metabolic activities. Hence, LLLT is involved primarily in modification of photoacceptors' function [9].

An important part of any light absorbing molecule is the chromophore. Chromophores are present in protein structures and have excitable electrons. When chromophore electrons absorb light they move from low energy ground state to a high energy excited state. This in turn changes the overall structure of the molecule resulting in changes in its function and cellular metabolism. The electron excitation in cells can either occur within a structure which has alternating single or double bonds or in open or closed pyrrole rings. Chromophores have a distinct property of absorbing particular wavelengths and reflecting the rest, giving color to molecules [9].

An important photoacceptor in humans that absorbs light in near infra-red region is hemoglobin. LLLT also has effect on certain other cells like fibroblasts and epithelial cells. As hemoglobin is found mainly in RBCs, it suggests that there are other photoacceptor molecules in the near infra-red range, most commonly myoglobin and COX, which produce LLLT effect [9].

Mitochondria is primarily involved in cellular metabolism and hence their density is very high in neurons in the brain due to high metabolic requirements. It is found that mitochondria are sensitive to light in red to near infra-red region. Light has various effects on mitochondria such as change in optical properties, increase in oxygen consumption and metabolism. The primary photoacceptor in the near infra-red region present in mitochondria is COX [9].

COX is the terminal electron acceptor in the mitochondrial respiratory chain and is responsible for 95% of oxygen consumption. COX has four redox metal centers: Cu_A, Cu_B, Hem a and Hem a_3 [11]. During the catalytic cycle electrons flow through these centers in a sequence reducing oxygen to water. The electron flow within COX is influenced differentially by electronic excitation of the four centers in different sequences. The enzyme can either be fully reduced or

fully oxidized or can be in intermediate state. It is this intermediate state in which COX is sensitive to LLLT and not when it is in fully oxidized or reduced states [9].

1.2.3 Mechanism of Action

LLLT has mainly two types of effects, namely primary and secondary.

a) Primary Effects

The primary effect is the direct changes taking place in the photoacceptor when it is excited by light. These effects occur only when target tissue is irradiated with light. There are three different primary effects. First, respiratory chain's components' redox changes. LLLT can either reduce or oxidize CCO and these changes result in changes in electron flow. In the presence of cytochrome c oxidase, there is an increase in oxidation of cytochrome c by LLLT. Consumption of oxygen and membrane potential of mitochondria also increases due to LLLT, activating the permeability pores of mitochondria [12, 13]. Second effect is the free radicals' generation, including superoxide ion generation by one electron auto-oxidation and singlet oxygen generated by photodynamic action. This effect indicates that the reactive oxygen species play an important role in cellular signaling apart from just being harmful by-products. Third, localized transient "heating" of the absorbing chromophore based on electric or light oscillations [12]. All molecules including water in the target tissue are affected by such oscillations due to their more general effect. LLLT can be said to strengthen hydrogen bonds and build networks of large-size hydrogen bonds that allow quick energy. Thus, LLLT can cause non-equilibrium electrical fluctuations that bias Brownian motion and induce mechanisms that support electron pumping without heat transfer [14].

b) Secondary Effects

The primary effects of LLLT are followed by its secondary effects and hence the secondary effects occur in the absence of light. These effect the homeostasis of the cells which include changes in enzyme functions. LLLT activates signaling pathway from mitochondria to nucleus which in turn causes adaptive responses. The initial stage of the pathway is characterized by an increase in NAD/NADH ratio and mitochondrial intermembrane potential, dissociation of nitric oxide from cytochrome oxidase and ATP pool modification. ATP activates P2 receptors which results in an increase in calcium release and cAMP [15]. Increase in ATP also leads to increase in gene expression. This cascade of changes result in an increase in mitogenic signals, surface molecular expression, energy metabolism and a decrease in inflammation and apoptosis. All these changes help in preserving neuronal structure and function such as visual, motor and cognitive functions [9].

High-energy electrons are fed into the mitochondrial respiratory chain by electron donors like $NADH^+$ or $FADH^+$, which interact with complex I/II. Electrons then flow to ubiquinone and subsequently to complex III. From complex III electrons then flow to cytochrome and finally reach cytochrome oxidase (complex IV). During the electron transfer, energy is released in a tightly regulated fashion, which allows pumping of protons into the mitochondrial intermembrane space, allowing the storage of energy as an electrochemical potential which is later used for ATP synthesis. LLLT improves cell respiration in that LLLT directly stimulates cytochrome c oxidase increasing its catalytic activity on ATP synthase resulting in an increase in ATP production. [36]

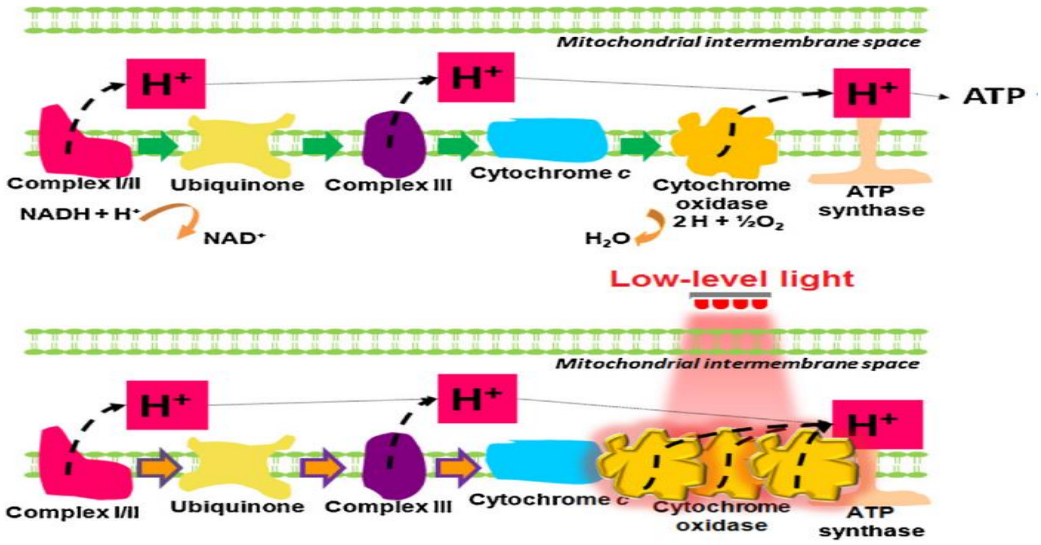


Figure 2: Mechanism of action of LLLT [36]

1.2.4 Effects on neurological tissues

Neurons have a demand for sustained aerobic energy production as they are highly specialized cells. Neuroplasticity, neuroprotection and electrophysiological functions, all are based on mitochondrial aerobic metabolism. Even for complex functions such as data integration, sensory processing, expression of memory and motor function activation require energy. Any impairment of mitochondrial metabolism would lead to neurological impairment, neuronal dysfunction and neurodegeneration [16]. Hence, improvement in mitochondrial metabolism would enhance the brain function.

1.3 Post-Traumatic Stress Disorder (PTSD)

Post-traumatic stress disorder is acquired by a person who faces terrifying ordeal that involved physical harm or threat of physical harm. PTSD is different than other traumas in that the reaction to traumatic events sustains and is extremely unlikely to get resolved without a treatment. Among those who develop trauma most do not develop PTSD and only 7% of the people develop PTSD.

Military combat is the most common trauma among men with PTSD. Combat-related PTSD is found in 9-25% of war zone veterans [17, 18, and 19] and is accompanied with other mental disorders [20], even after the veterans return to their civilian lives.

1.3.1 Diagnosis

A person should have been exposed to either of the below types of traumas to be diagnosed for PTSD according to DSM-5 [39]. The traumas include: -

a. Experiencing a traumatic event directly
b. Witnessing, in person, an event that happened to someone else
c. Learning about a violent or unexpected death of a friend or family member, however, natural death of family members does not qualify.
d. Experiencing repeated or extreme exposure to adverse details of traumatic events.

Exposure to electronic media like photographs, videos or television cannot be considered as a traumatic event according to DSM-5 [39].

A person must have a combination of symptoms to be diagnosed for PTSD and should include 1 re-experiencing, 1 avoidance, 2 negative alterations in cognitions and mood and 2 hyperarousal symptoms. These symptoms should last for at least a month. PTSD is usually accompanied with substance abuse, depression or anxiety disorders [39].

1.3.2 Symptoms

a) Re-Experiencing Symptoms

The re-experiencing symptoms interfere with a person's daily routine. Re-experiencing may be triggered by situations, objects and even words that are reminders of the event. The symptoms

include: recurrent distressing recollections of event or dreams of event, acting or feeling as if event is recurring(flashbacks), psychological distress to cues resembling event and physiological reactions like sweating and increased heart rate to cues resembling events [39].

b) Avoidance Symptoms

A person with PTSD tries to avoid things that remind them of the traumatic event due to which they change their personal routine. The symptoms include: avoidance of thoughts or feelings that remind them of the event, avoidance of activities, people, places or conversations that remind them of the event [39].

c) Negative alterations in cognitions and mood

The numbing symptoms in DSM-4 include: diminished interest or participation in significant activities such as social events, feeling detached from others, inability to experience positive emotions, inability to recall important aspects of the trauma. Negative emotional state and exaggerated negative beliefs or expectations, blaming oneself or others were included in DSM-5 [39].

d) Hyperarousal symptoms

These symptoms are constant and are not triggered by any reminders of the event. The symptoms include: sleep disturbance, irritable behavior and outbursts of anger, problem in concentrating, hypervigilance, reckless or self-destructive behavior and exaggerated startle response [39].

1.3.3 Treatment

PTSD treatment includes psychotherapy, medication or both.

a) Pharmacological treatment

The first line of treatment includes antidepressants, sertraline and paroxetine. They can act on symptoms like anger, sadness and worry. Even serotonin-norepinephrine reuptake inhibitors are also recommended. The antidepressants may have few side effects such as: headache, sleeplessness or drowsiness, nausea, agitation and sexual problems. Most of these side effects last only for a few days or weeks. Other medicines include benzodiazepines, antipsychotics and antidepressants like fluoxetine and citalopram [39].

b) Psychotherapy

It is also known as “talk therapy” wherein the patient speaks to a mental health professional to treat the illness. In this therapy the patients share their experiences, things that they have faced or are facing and even the improvement in their personal and professional lives either one-on-one or in a group. Various types of psychotherapy help people with PTSD, in which, some may act directly on the symptoms of PTSD whereas the others act on personal or professional problems [39].

One such psychotherapy which has been found to be effective in treating PTSD is the Cognitive Behavioral Therapy (CBT). CBT helps in building new cognitive skills as well as involving in new behaviors or changing the existing ones.

One such Cognitive Behavioral therapy used for treating PTSD is Prolonged Exposure Therapy. Prolonged exposure therapy helps people face and overcome their fears and involves making the patient to relive the trauma and confront them, by repeatedly exposing them to thoughts, feelings and situations related to the trauma either by visiting the place where traumatic event occurred or by showing them visuals or by asking them to imagine the event or asking them to explain the event in writing. This helps the patient to accept the traumatic event rather than just avoiding it. This therapy has basically four stages namely: educating the patient about PTSD, the effects of trauma and the reason why they are exposed to the memories related to the event, second stage involves teaching them breathing skills to manage anxiety that they might face while recalling the event, third and fourth stage involves live and imaginal exposure where the person is exposed to the events. The patients are exposed until their stress levels, during the recall phase, reduces significantly [39].

Another therapy used for treating PTSD is Cognitive Processing Therapy, wherein the patient's negative view about the event is changed. They usually remember or see those events in a way that they might feel guilty about themselves, like the war veterans for killing people in the war, or make them feel ashamed of themselves, like a rape victim might detach themselves from the society being ashamed of what has happened with them. This therapy has four stages. In the first stage the patient is educated about PTSD and its symptoms. Second stage involves processing the trauma wherein they are asked to explain their interpretation of the trauma and their view point about the trauma. In the third stage they build skills to challenge the thoughts and in the fourth they implement these cognitive skills on their thoughts of the event and restructure them changing their view about the event [39].

1.3.4 Effects of PTSD on neurocognitive function

Numerous neuropsychological studies have identified cognitive dysfunctions associated with PTSD, such as memory impairments, attention deficits and learning disabilities. Patients with PTSD have significant reductions bilaterally, both in the volume of the hippocampus and amygdala of the temporal lobes and of the frontal cortex. Consistent with the effects of stress on brain structures that mediate memory, PTSD is associated with a wide range of memory deficits [35]. PTSD patients show deficits in declarative memory, enhanced responses to conditioning, and perseverative errors (possibly related to frontal lobe dysfunction) [34].

1.4 Aims of this study

The aim of this study was to evaluate the effect of transcranial LLLT on prefrontal cognitive functions, namely attention, vigilance and memory, of PTSD veterans using a high performance fNIRS system. In this study we measured both behavioral responses as well as hemodynamic changes occurring while performing two neurocognitive tasks. We first carried out measurements on healthy controls to get a standard response, both behavioral and hemodynamic, without giving a laser treatment. We then carried out measurements on PTSD subjects, where each of the four subjects underwent six laser treatments. They were asked to perform task before and after the treatment during each treatment session in order to evaluate the effect on cognitive functionality over six treatment sessions and also to evaluate the pre and post treatment effects on the same. We also compared the behavioral response and hemodynamic response between healthy controls and PTSD subjects, before receiving the first treatment and also after receiving the last treatment, in order to evaluate their performance with respect to controls and also to evaluate if the treatment had any beneficial and visible effects on the cognitive functionality of the PTSD veterans. This

was done by associating behavioral and hemodynamic results in order to understand the neural activation patterns.

1.5 Outline of this Thesis

Chapter 1 consists of brief introduction of fNIRS, low level laser therapy (LLLT) and Post-Traumatic Stress Disorder (PTSD). Chapter 2 describes the experimental methodology. This chapter briefly describes the selection criteria for healthy controls and PTSD subjects, the instrumentation used, explains in detail about the neurocognitive tasks, the experimental protocols used for controls and PTSD subjects and the data analysis methodology used to analyze the results. The results, for behavioral scores and hemodynamic response, obtained by comparing the data within the PTSD subjects as well as between controls and PTSD subjects is described in chapter 3. Chapter 4 discusses the results obtained and, the limitations and future work of the study.

Chapter 2

Methodology

This section describes the experimental methodology used for this study. The controls were not given any treatment and were just asked to perform PVT and DMS tasks. PTSD subjects on the other hand underwent six laser stimulations over 3 weeks, twice in a week, and were asked to perform both the tasks before and after the treatment, for each of the six treatment sessions.

2.1 Subjects and Eligibility criteria

The experiment was conducted under the protocol that was approved by The University of Texas at Arlington's Institutional Review board.

2.1.1 Eligibility criteria for healthy controls

Young healthy adults of age ranging from 22 to 40 years of any ethnic background were considered for the study. Potential participants were screened initially with a questionnaire to exclude those who had history of any psychiatric illness, history of severe TBI or intake of any medication at the time of study [21]. The participants were selected from the local community of the University of Texas at Arlington through one to one solicitation.

Each participant was orally guided through the consent form on the day of the experiment by the investigator and participants were allowed to ask questions after reading through the form. Before every experiment was started, a signed consent form was obtained. Participant's demographics such as age and gender was recorded.

2.1.2 Eligibility criteria for PTSD Veterans

Combat-exposed veterans who were preparing for or enrolled in higher education were recruited via a supported education intervention clinical trial conducted by the mental health translational research center of the school of Social Work at the University of Texas at Arlington [21]. Veterans with medical records confirming prior diagnosis with PTSD, were experiencing clinically significant distress and functional impairment affecting their cognitive and related academic performance at the time of study were referred for the experiment. An experienced licensed clinician confirmed the PTSD diagnosis and comorbid conditions prior to the experiment using Structured Clinical Interview for DSM disorders-research version (SCID-RV) [22] and scores on three self-report questionnaires namely, PTSD checklist- military version (PCL-M) [23,24], Mississippi scale for combat-related PTSD [25,26], and the PTSD subscale of Minnesota Multiphasic Personality Inventory [27]. For the veterans to be enrolled for the experiment, they were required to have at least one of the three self-report scores to be above its respective clinical cutoff values (i.e., PCL-M > 50, Mississippi scale > 130, or PTSD subscale > 30), besides their prior diagnosis and current diagnostic evaluation confirmation of PTSD and comorbid conditions [21].

2.2 Instrumentation

2.2.1 Treatment Laser

This treatment consisted of applying light of wavelength 1064nm (which falls in the near infra-red region), using a laser diode supplied by Cell Gen Therapeutics LLC (Model CG-5000 laser, HD Laser Center, Dallas, TX, USA) (Figure 3). Although this device is not FDA approved for this specific study, it has been approved by FDA for various other uses on humans which are safe, such

as for stiffness in arthritis, circulation improvement, muscle and joint pain temporary relief, muscle spasm and muscle tissue relaxation [28]. The laser received approval from the University of Texas at Arlington Laser Safety Program and a standard operating procedure for the laser was approved by the University Laser Safety Officer.

The laser was used at an irradiance of 250 mW/cm^2, as well as the energy density used was 60 J/cm^2, and are the same parameters that showed to have beneficial psychological effects [28, 29]. The laser had a diameter of 4 centimeters. At the described level of power used, CG-5000 emits low energy, its exposure to tissues is harmless and causes negligible heat and no physical damage. Cell Gen Therapeutics uses similar settings for treating lower back pain, migraine headaches and sciatica [29].

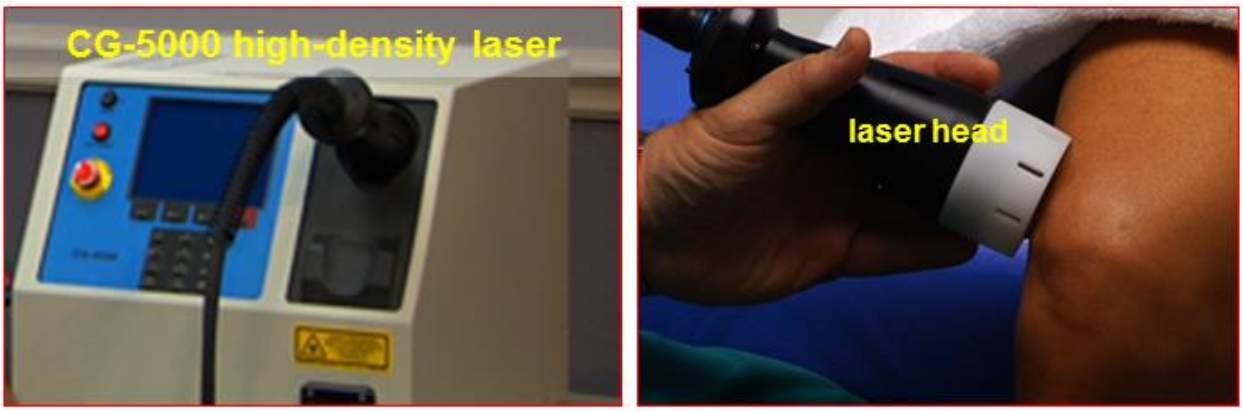


Figure 3: The CG-5000 high-density laser system (left) and laser head (right)

2.2.2 FNIRS System

The hemodynamic activity, while performing the task, was acquired for each participant by a high performance fNIRS system (Cephalogics LLC, Boston, MAA) [30]. Light emitting diodes operated at two wavelengths namely 750 and 850 nm were used as light sources by the system and the detectors used were avalanche photodiodes. The data was sampled at the rate of 10.8 Hz.

Figure 4 shows the fNIRS probe geometry used for this study. The geometry consists of 12 light sources and 16 detectors (8 detectors and 6 sources on each hemisphere). The probe was placed bilaterally and symmetrically on the participant's forehead. The bottom line of 6 light sources in the probe was just above the eyebrows and its midpoint was about 3.5 cm in distance from the nasion [21]. The fNIRS probe provided a total of 36 channels when only the nearest source-detector pairs were considered (the nearest source-detector distance was 2.5 cm). Other large source-detector distances were not included because their signals were too weak to be scientifically meaningful. The probe assembly was constructed with low-weight optical fibers (TechEn Inc., Boston, MA) and thin polyethylene film to ensure participants' comfort during the experiment [21].

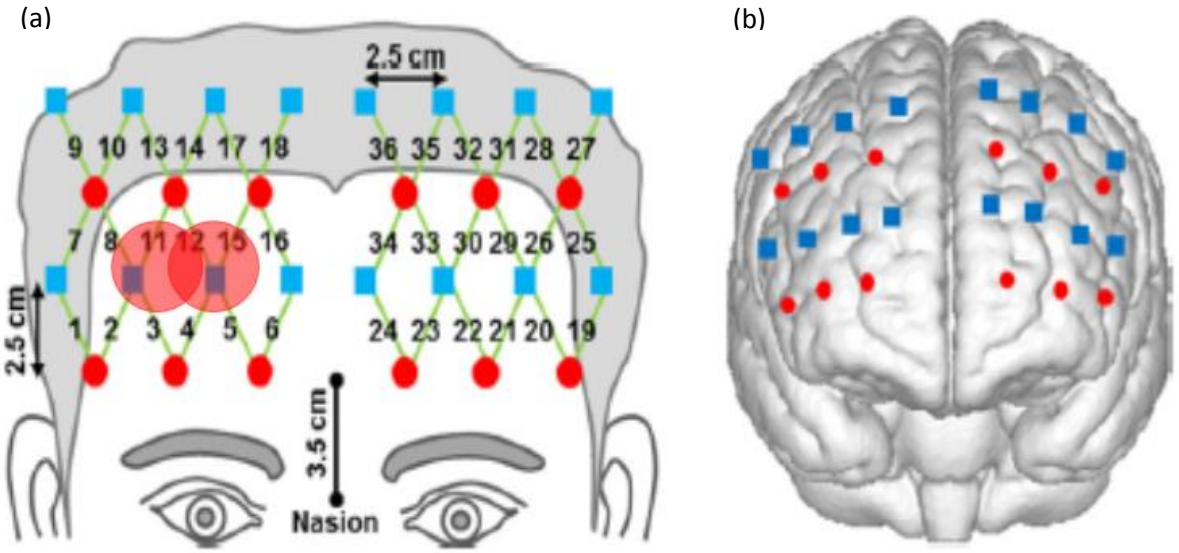


Figure 4: (a) Configuration of the fNIRS probe showing the treatment site. Red circles represent light sources, blue squares represent detectors, and green lines represent the nearest source-detector pairs (channels) to measure the brain activities. (b) Co-registered positions of the optodes on a standard brain atlas

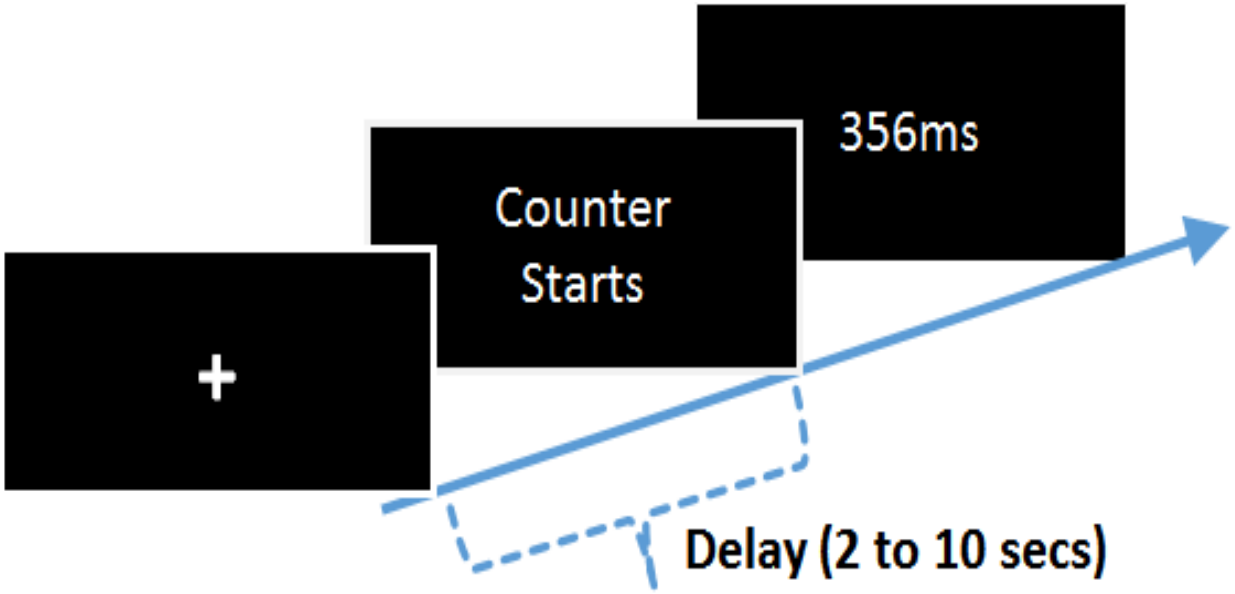
2.3 Computerized Neurocognitive tests

The two neurocognitive tasks namely Psychomotor Vigilance Task (PVT) (for testing attention or vigilance) [31] and Delayed Match-to-Sample (DMS) task (for testing short term memory) that were used in this study were reported to be published in a paper by Gonzalez Lima et al. Psychology Experiment Building Language (PEBL), an open source programming language, was used to implement PVT and DMS tasks. One desktop computer in the lab was designated as the testing apparatus. In the PVT test, the participant looks at a small '+' fixation point which appeared briefly at the center of the computer screen. After the fixation point disappeared, a black screen is presented for a random interval of 1 to 10 seconds. Then, a bright millisecond timer appears in the center of the screen. Participants were instructed to respond via button press as rapidly as possible, upon detection of the counter stimulus, in order to stop the counter from updating. The final counter value corresponds to the participant's reaction time, which was displayed for 1 second, providing feedback for that particular trial. Participants were given 30 seconds to make a response before the trial was aborted. The data gathered during the task included each trial's intertribal interval, reaction time in milliseconds and a code number indicating that the trial was a success i.e. response in less than 30 seconds), a lapse i.e. no response in 30 seconds, or a false alarm i.e. response with button press prior to the start of the timer. All these information was stored by the computer for later analysis. The PVT task is illustrated in figure 5 (a).

The DMS task measures reaction time as well as the accuracy. The DMS task is delineated in figure 5 (b). The participant viewed a 4 × 4 grid of brightly colored squares with a unique, randomly-generated pattern for each of 30 trials. The 16 squares grid consisted of 7, 8 or 9 red-colored squares, with yellow squares comprising the rest of the grid. Once the participant memorizes the pattern, with a key press the stimulus grid disappears and the screen remains blank

for 3.5 seconds. After this delay, two stimuli were then presented on screen (a “match” and “non-match”). The match was identical to the previous stimulus, while the “non-match” contained 1-2 randomly switched squares. The participant would register their selection by a key press. “Correct” or “Incorrect” was displayed for one second after each trial to provide feedback. Correct/Incorrect status and memory retrieval latency (reaction time) for each trail were stored in the computer for later analysis. The inter trial interval was set to be 3.5 seconds.

(a)



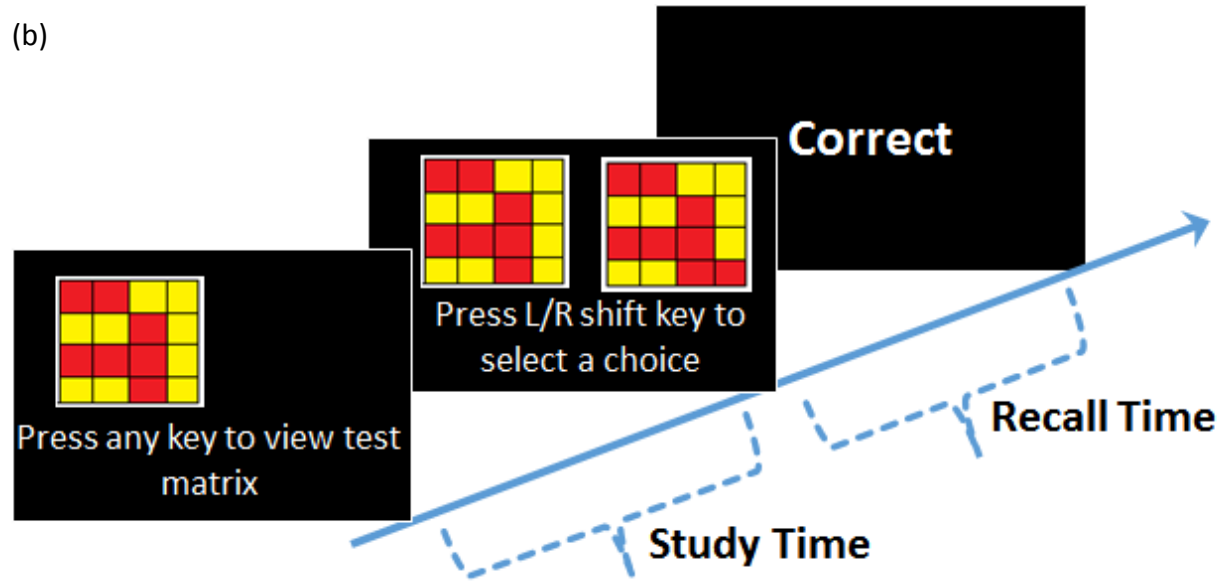


Figure 5: Pictorial representation of the events comprising of (a) PVT and (b) DMS tasks

2.4 Experimental Procedures

2.4.1 Controls

The participants were asked to sit comfortably. The participants were given a 1-minute trial for each of the two tasks, PVT and DMS, in order to familiarize themselves with the tasks. A brief instruction was given before each of the trial tasks. The fNIRS probe was then placed on the head as shown in figure 4(a) and as described in section 2.2.2. In order to get better signal, any hair under the sources and detectors were split out. After this, the participant was given a PVT block of 40 trials, which lasted for approximately 5 minutes. The intertrial intervals were randomly chosen from between 2 to 10 seconds and hence the average intertrial interval was around 6 seconds [28]. The participants were then asked to take part in the DMS task which consisted of 30 trials, that was approximately 5 minutes long. The participants were informed that while there was no time limit for either studying the target or choosing the match, they should try to be "as fast as

possible, while being accurate at the same time" [28]. The participant's behavioral scores, for both PVT and DMS tasks, were recorded in the same way as the previous study by Barrett et al. The fNIRS data was acquired 10 seconds prior to the experiment and ceased immediately after the tasks were finished. Laser stimulation was not administered to the controls. The entire experiment lasted for ~20 minutes.

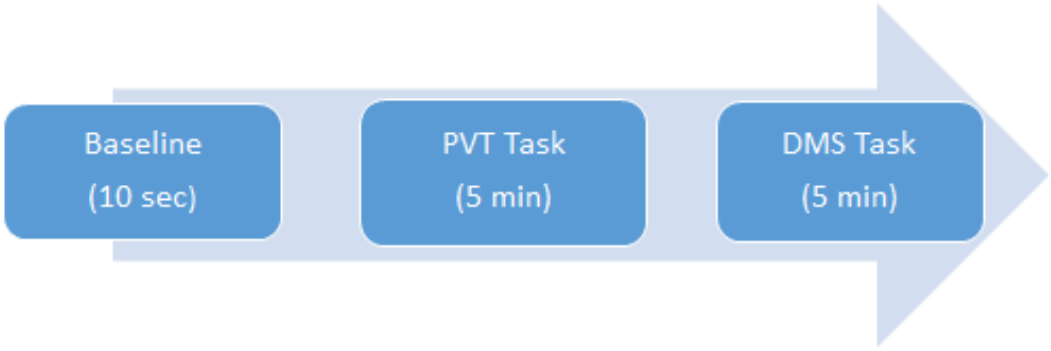


Figure 6: Experimental protocol for controls

2.4.2 PTSD Veterans

The experiment on PTSD subjects consisted of three phases 1) pre-treatment tests, during which the participants were asked to perform PVT and DMS tasks. 2) Transcranial LLLT, in which the participants received laser treatment that was administered on their right frontal lobe. 3) Post-treatment tests, during which the participants were asked to perform PVT and DMS tasks once again. The entire experiment lasted for ~35-40 minutes. The participants received two treatment sessions per week and hence, six treatment sessions were completed in a span of 3 weeks.

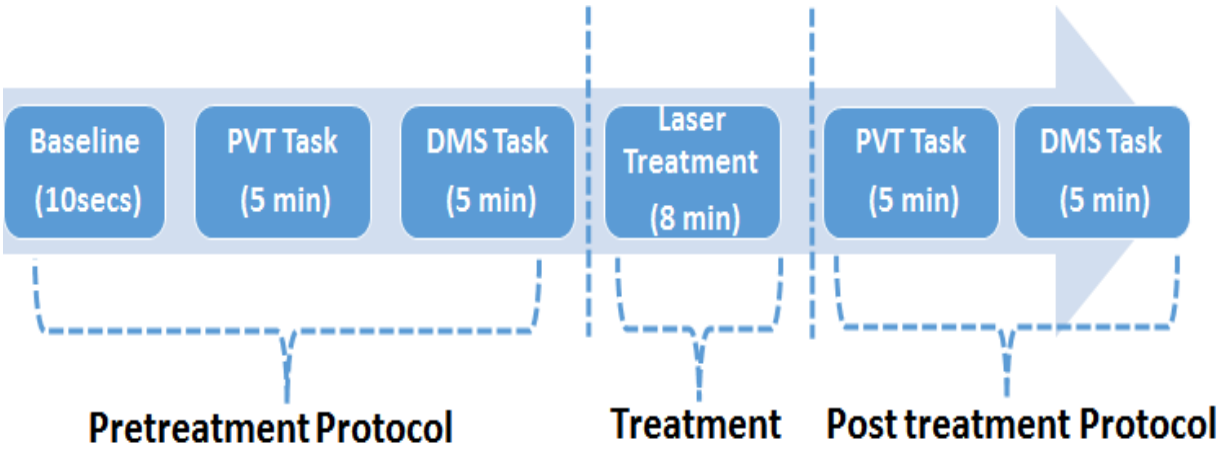


Figure 7: Experimental protocol for PTSD veterans

2.4.2.1 Pre-Treatment Protocol

The participants were asked to sit comfortably. The participants were given a 1-minute trial for each of the two tasks, PVT and DMS, in order to familiarize themselves with the tasks. A brief instruction was given before each of the trial tasks. The fNIRS probe was then placed on the head as shown in figure 4(a). In order to get better signal any hair under the source and detectors were split out. After this, the participant was given a PVT block of 40 trials, which lasted for approximately 5 minutes. The intertrial intervals were randomly chosen from between 2 to 10 seconds and hence the average intertrial interval was around 6 seconds [28]. The participants were then asked to take part in the DMS task which consisted of 30 trials, that was approximately 5 minutes long. The participants were informed that while there was no time limit for either studying the target or choosing the match, they should try to be "as fast as possible, while being accurate at the same time" [28]. The participant's behavioral scores, for both PVT and DMS tasks, were recorded in the same way as the previous study by Barrett et al. The fNIRS data was acquired 10 seconds prior to the experiment and ceased immediately after the tasks were finished.

2.4.2.2 Laser Treatment

Once the participants completed the PVT and DMS tasks they were administered with transcranial LLLT. The participant and the experimenter were locked in a room, with no reflective surfaces and black walls, during the laser stimulation. While the laser was being used, a warning sign on the outside of the room was switched on. All the individuals present in the room wore protective eyewear (900–1000 nm: 5+, 1000– 2400 nm: 7+; 2900–10600 nm: 7+). Precaution was although taken, to not shine light on the eye during the laser stimulation. Participants were asked to keep their eye closed in addition to the eyewear. The parameters of the laser were set as described in section 2.2.1. An internal mechanism automatically calibrates the power output of the laser. The laser was focused at the right frontal pole of the cerebral cortex, associated with sustained attention and memory, which is the right frontal cortex's most anterior region. The FP2 point was the center for the forehead stimulation site, which is in reference to the EEG electrode placement used in the 10-20 system [39]. Medially, the stimulation site extended for about 4 cm diameter area from FP2 point and laterally, it extended for another 4 cm diameter area from the same point and is as shown in figure 4(a) [39]. The treatment was given for a total of 8 minutes and was divided into eight one-minute cycles. The on/off of each cycle was controlled by the experimenter. Each cycle was counted down, marked by a timer and with a beep from the laser system. Each participant received four one-minute treatments on each of the two sites on the right forehead, alternating between sites that were medial and lateral to the FP2 point. The laser stimulation protocol used is as shown in figure 8.

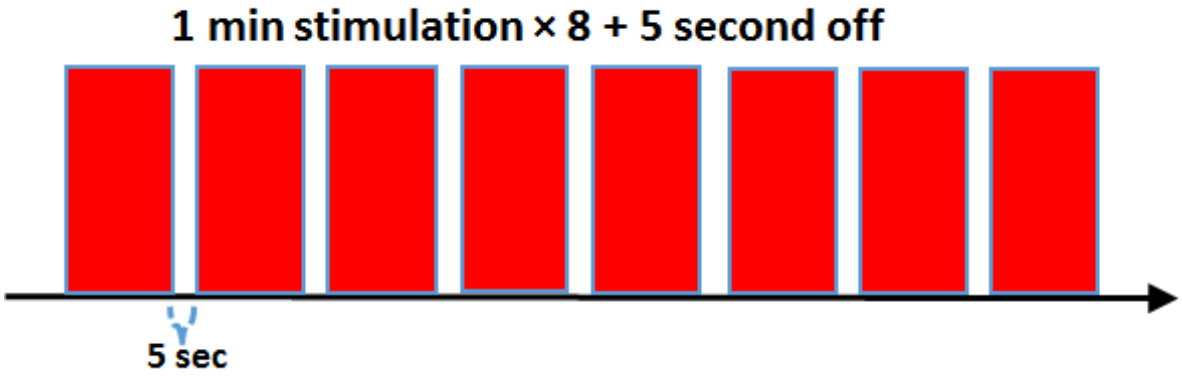


Figure 8: LLLT stimulation Protocol

2.4.2.3 Post-Treatment Protocol

Participants were asked to repeat the DMS and PVT tasks after the transcranial LLLT. A comparison between the controls and PTSD subjects (before the first laser stimulation and after the last laser stimulation) will allow us to know the effect of treatment on PTSD subjects. Also, evaluation of effects, on neurocognitive functions (attention and memory), of laser stimulation over six treatment sessions and also a comparison between pre and post treatment was performed.

2.5 Data Analysis

2.5.1 Data Screening and Pre-Processing

A publically available toolbox called HomER [32] was used to screen and process each measurement session's fNIRS data. The raw data were visually inspected to exclude trials which had significant data discontinuities. Trials with signal swings of 15% or more from the baseline intensities were excluded as they are attributed usually to motion artifacts during the experiment [21]. The qualified data was then band pass filtered to remove hemodynamic fluctuations due to breathing and arterial pulsations. The data was low pass filtered at a cut-off frequency of 0.4Hz in

order to remove fast-oscillating cardiac waves and electronic noise and high pass filtered at a cut-off frequency of 0.02Hz in order to remove possible slow baseline drift [21]. The changes in hemoglobin concentration, were calculated relative to the baseline suing MBLL. The temporal profiles were then averaged over the remaining trials in each session in order to obtain the individual hemodynamic responses that were evoked by the PVT and DMS tasks.

2.5.2 Topography and visualization

An optical topography toolbox called EasyTopo [33], was used to generate topographic maps of prefrontal activations. EasyTopo is based on a standard brain MRI atlas and implements 2D angular interpolation of channel-wise data in a spherical coordinate system. In this study, channel-wise block averaged HbO and Hb concentrations were interpolated to generate activation maps.

2.5.3 Statistical Analysis

The statistical analysis was performed on the PTSD subject's data over six treatments to evaluate the treatment effect. Also, a comparison was made between the controls and the PTSD subject's pre-treatment 1 (pre-LLLT 1) data and between controls and the PTSD subject's post treatment 6 (post-LLLT 6) data, in order to evaluate the neurocognitive functions of PTSD subjects prior to receiving any treatment (before the first treatment) and after the last (sixth) treatment session.

2.5.3.1 Behavioral Scores

For comparison between controls and PTSD subjects' data, for both pre-LLLT 1 and post-LLLT 6, non-parametric Wilcoxon rank sum test was used. We also used Wilcoxon rank sum test to compare pre-LLLT 1 and post-LLLT 6 in order to evaluate the effect of treatment over six sessions. As the data in this study cannot be assumed to be normally distributed due to small sample size we used this test as an alternative to t-test.

2.5.3.2 Hemodynamic Measure

The hemodynamic response was analyzed by using the block average channel-wise HbO and Hb concentrations. For this study we only analyze the block averaged channel-wise HbO concentrations.

The channel-wise block averaged [HbO] changes were calculated for both controls and PTSD subjects. For controls, we then selected channels with significant activation with respect to baseline in order to select the region of interest. The corresponding channels in the ROI were then selected for the PTSD subjects. Each of the channels were then integrated over time.

The integrated [HbO] changes were then compared between controls and PTSD, for both pre-LLLT 1 and post-LLLT 6 and also within PTSD subjects, between pre-LLLT 1 and post-LLLT 6. The comparisons were made within each side, right and left, of the head.

A two-sample two-tailed t-test was used to compare between controls and PTSD subjects and a paired two-tailed t-test was used for comparing between pre-LLLT 1 and post-LLLT 6.

Chapter 3

Results

This section consists of the experimental results obtained from the analysis based on the methodology mentioned in the previous section.

3.1 Behavioral Scores

3.1.1 Controls

For this study we had a total of 8 normal healthy control subjects. The demographics of the subjects are as shown in the table 2.

Table 3 shows the average reaction time, study time, recall time and accuracy for the controls.

Table 2: Demographics of healthy controls

Name	Age	Gender
1	26	Male
2	24	Male
3	26	Male
4	25	Male
5	24	Male
6	25	Male
7	24	Male
8	40	Male

Table 3: Average behavioral scores for PVT and DMS task

PVT	DMS		
Reaction Time (ms)	Study Time (sec)	Recall Time (sec)	Accuracy
339.98	2.02	1.33	29

3.1.2 PTSD

The results of Wilcoxon rank sum test to compare pre-LLLT 1 and post-LLLT 6 are shown in Table 4.

Table 4: Results of Wilcoxon rank sum test for PTSD subjects

Task	PTSD(Pre-LLLT 1)		PTSD (Post-LLLT 6)		p-value
	Sum of Ranks	Mean Rank	Sum of Ranks	Mean Rank	
PVT	18	4.5	18	4.5	1.114
DMS (Study Time)	26	6.5	10	2.5	0.014
DMS (Recall Time)	25	6.3	11	2.8	0.029
DMS (Accuracy)	20.5	5.1	15.5	3.9	0.543

For PVT, there is no significant difference ($p<0.05$) seen in the reaction time between pre-LLLT 1 and post-LLLT 6, from Table 4. Figure 9 shows the plot of average reaction time for both pre and post treatment over six treatment sessions, for the four PTSD subjects. From the plots we can see that there is no much change in the reaction times over 6 treatments.

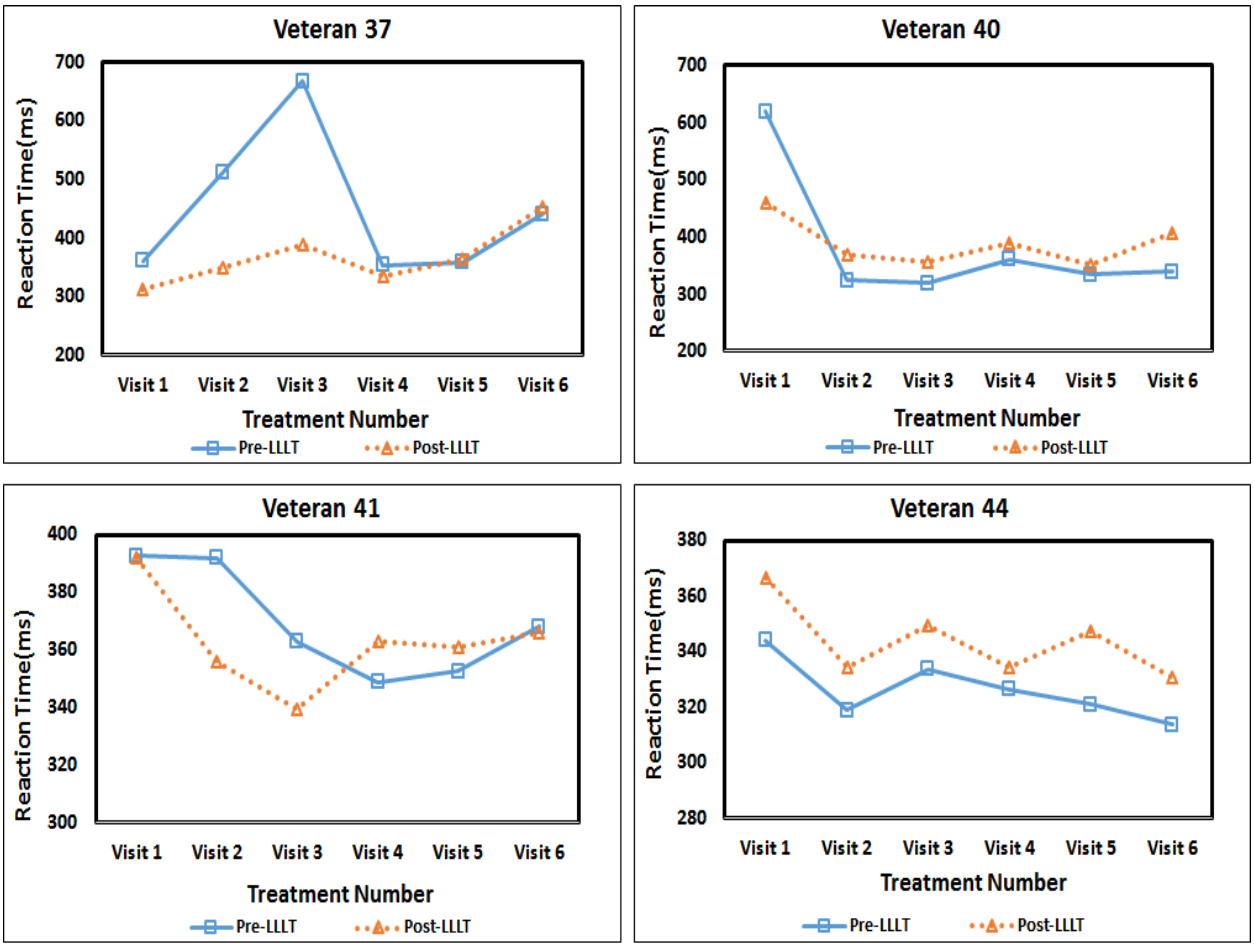
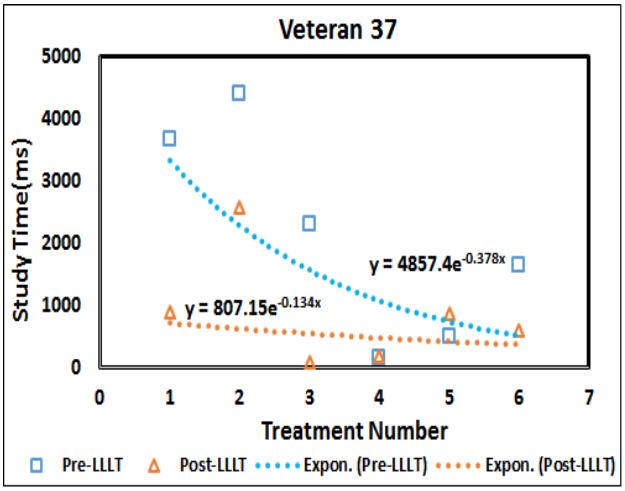
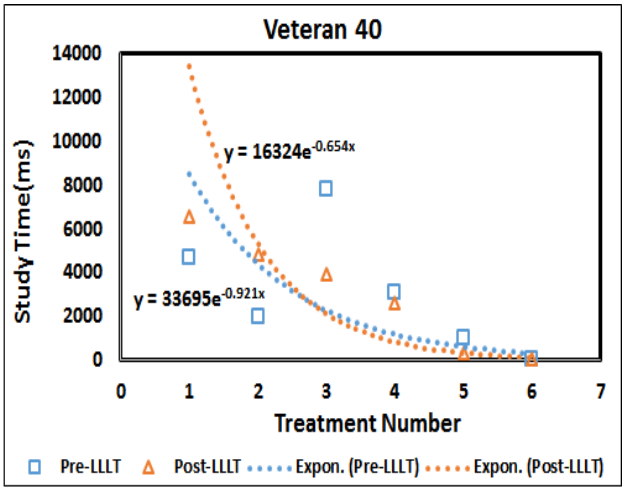


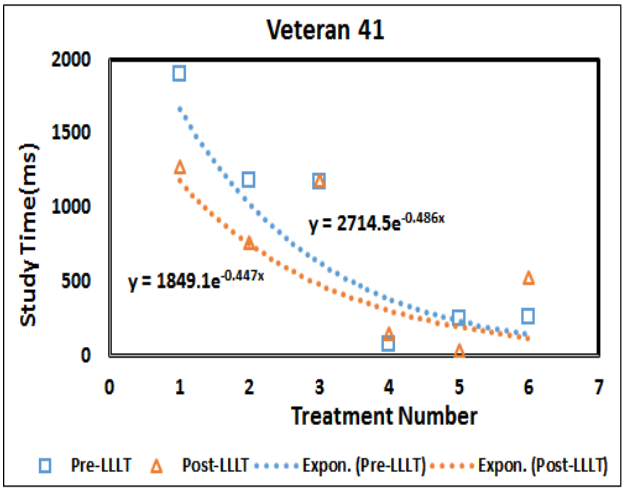
Figure 9: PVT average reaction time plots for the PTSD veterans

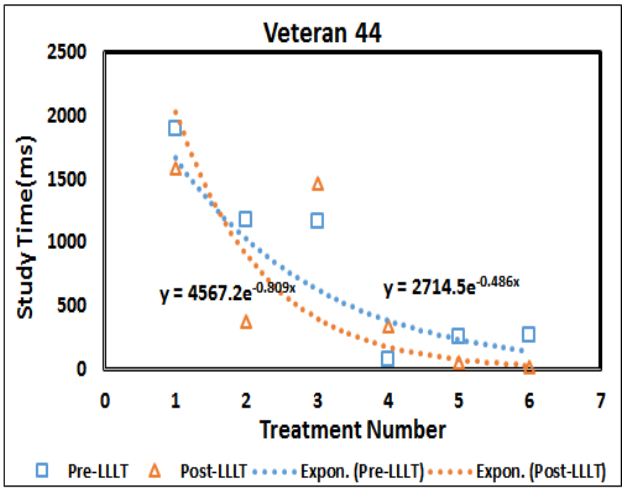
For DMS, the results in table 4 shows significant difference ($p<0.05$) between pre-LLLT 1 and post-LLLT 6 for study and recall times and shows no significant difference for accuracy. This is in agreement with the plots shown in figure 10(a), (b), and an overall decline in the study time and recall time is observed. We also observed that the study and the recall time seem to reach a plateau on the sixth treatment suggesting that a threshold is reached and there might be no further decline. However, the accuracy, shown in figure 10(c), does not change much over six treatments.

(a)

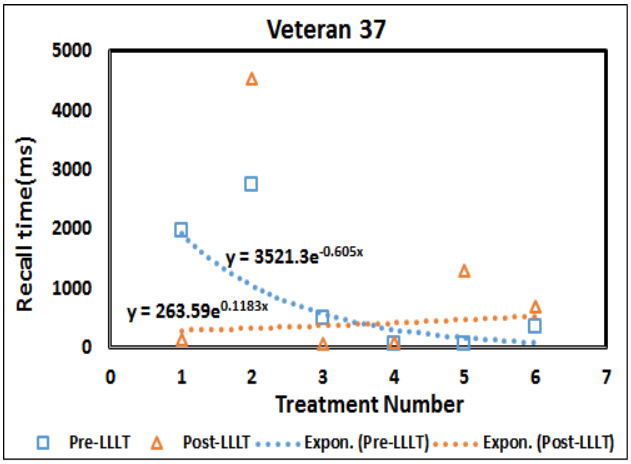

Veteran 37
Study Time(ms)
Treatment Number
y = 4857.4e-0.378x
y = 807.15e-0.134x
Pre-LLLT
Post-LLLT
Expon. (Pre-LLLT)
Expon. (Post-LLLT)

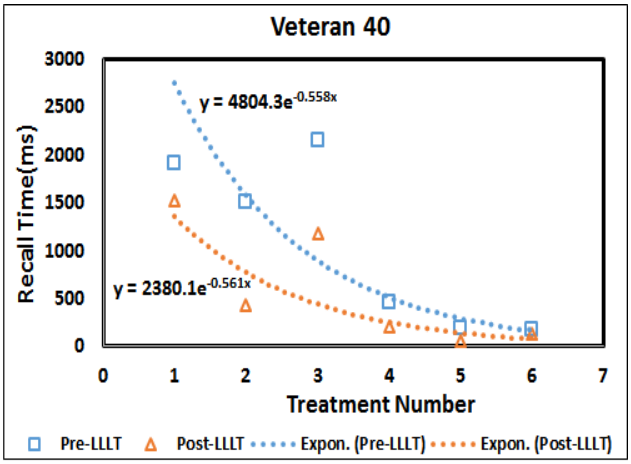

Veteran 40
Study Time(ms)
Treatment Number
y = 16324e-0.654x
y = 33695e-0.921x
Pre-LLLT
Post-LLLT
Expon. (Pre-LLLT)
Expon. (Post-LLLT)

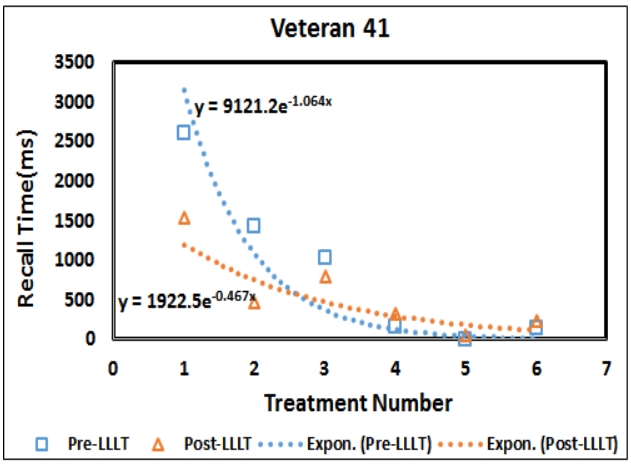

Veteran 41
Study Time(ms)
Treatment Number
y = 2714.5e-0.486x
y = 1849.1e-0.447x
Pre-LLLT
Post-LLLT
Expon. (Pre-LLLT)
Expon. (Post-LLLT)

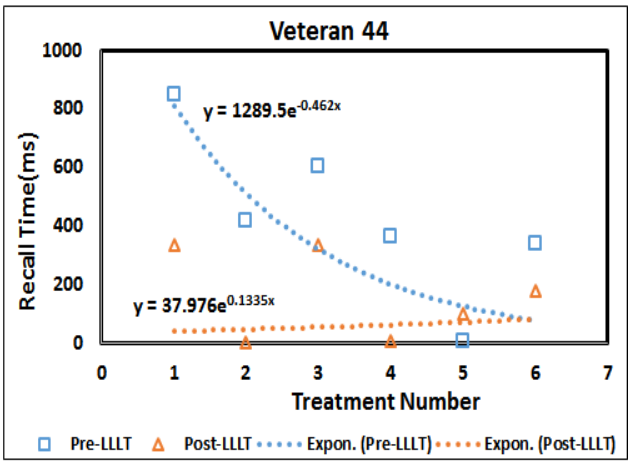

Veteran 44
Study Time(ms)
Treatment Number
y = 4567.2e-0.809x
y = 2714.5e-0.486x
Pre-LLLT
Post-LLLT
Expon. (Pre-LLLT)
Expon. (Post-LLLT)

(b)


Veteran 37
Recall time(ms)
y = 3521.3e-0.605x
y = 263.59e0.1183x
Treatment Number
Pre-LLLT
Post-LLLT
Expon. (Pre-LLLT)
Expon. (Post-LLLT)


Veteran 40
Recall Time(ms)
y = 4804.3e-0.558x
y = 2380.1e-0.561x
Treatment Number
Pre-LLLT
Post-LLLT
Expon. (Pre-LLLT)
Expon. (Post-LLLT)


Veteran 41
Recall Time(ms)
y = 9121.2e-1.064x
y = 1922.5e-0.467x
Treatment Number
Pre-LLLT
Post-LLLT
Expon. (Pre-LLLT)
Expon. (Post-LLLT)


Veteran 44
Recall Time(ms)
y = 1289.5e-0.462x
y = 37.976e0.1335x
Treatment Number
Pre-LLLT
Post-LLLT
Expon. (Pre-LLLT)
Expon. (Post-LLLT)

(c)

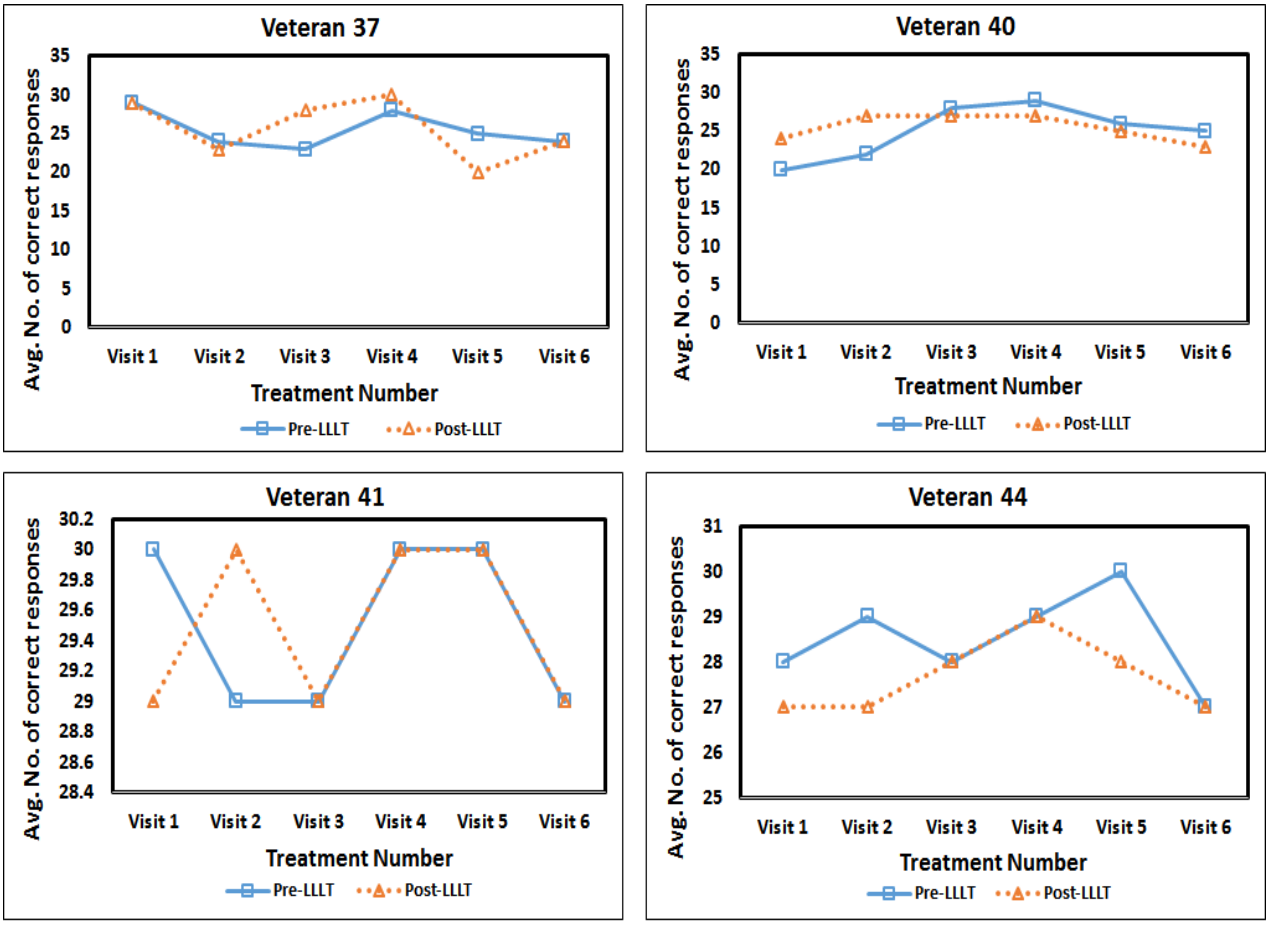


Figure 10: Plots of pre and post treatment average study time (a), recall time (b) and accuracy(c) for PTSD subjects over six treatments

3.1.3 Comparison between Controls and PTSD

Two comparisons were carried out between the controls and PTSD subjects. First, the reaction time, study time, recall time and accuracy were compared between controls and PTSD subjects before they received the first treatment i.e. pre-LLLT 1. Second, the comparison was made between the controls and the PTSD subjects after receiving the last (sixth) treatment i.e. post-

LLLT 6. The Wilcoxon rank sum test results, consisting of sum of the ranks and mean ranks for the two groups, for the two types of comparisons are as shown in table 5 and 6.

For PVT, the results of Wilcoxon rank sum test indicated that there is no significant difference between the two groups for both types of comparisons, suggesting that the performance of PTSD subjects, on the PVT task, did not improve after the six treatments.

For DMS task, in the first comparison, although there was no significant difference seen in the accuracy between the two groups there was a significant difference ($p<0.05$) in the study and recall times. It indicated that there was a significant difference between the performances of controls and PTSD subjects before receiving the first treatment. However, in the second comparison, there was no significant difference between the two groups in study time, recall time and accuracy, indicating that the PTSD subjects' performance on the tests had improved after receiving the six treatments.

Table 5: Results of Wilcoxon rank sum test of Behavioral scores for Control Vs. PTSD (Pre-LLLT 1)

	PTSD (Pre-LLLT 1)		Control		p-values
	Sum of Ranks	Mean Rank	Sum of Ranks	Mean Rank	
PVT	38	9.5	40	5	0.048
DMS (Study Time)	42	10.5	36	4.5	0.004
DMS (Reaction Time)	41	10.3	37	4.6	0.008
DMS(accuracy)	24	6	54	6.8	0.909

Table 6: Results of Wilcoxon rank sum test of Behavioral scores for Control Vs. PTSD (Post-LLLT 6)

	PTSD (Post-LLLT 6)		Control		p-values
	Sum of Ranks	Mean Rank	Sum of Ranks	Mean Rank	
PVT	36	9	42	5.3	0.109
DMS (Study Time)	24	6	54	6.8	0.808
DMS (Reaction Time)	36	9	42	5.3	0.109
DMS(accuracy)	15	3.8	63	7.9	0.076

3.2 Hemodynamic Response

A total of 14 channels were found to be significant for the DMS task and 12 channels for the PVT task. Figure 10 shows the significant channels which represented region of interest.

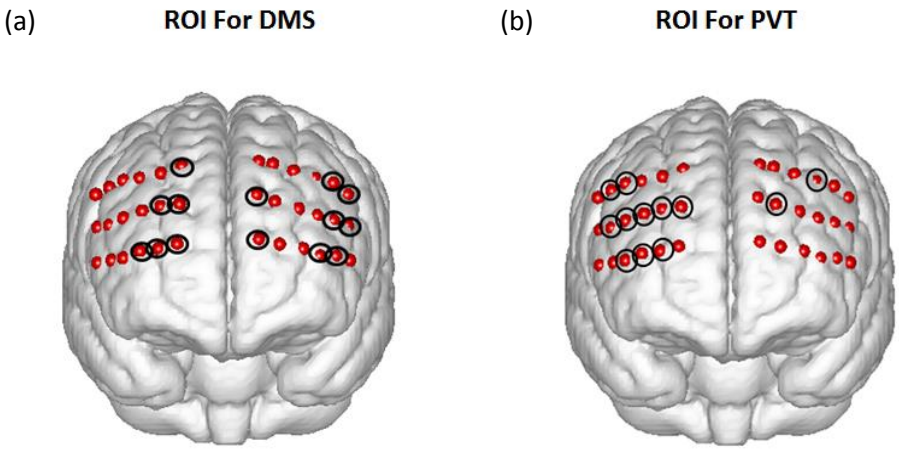


Figure 11: Region of interest for (a) DMS and (b) PVT. Significant channels are encircled in black.

3.2.1 Controls

Figure 12 shows the plots of block-averaged channels in the region of interest for controls.

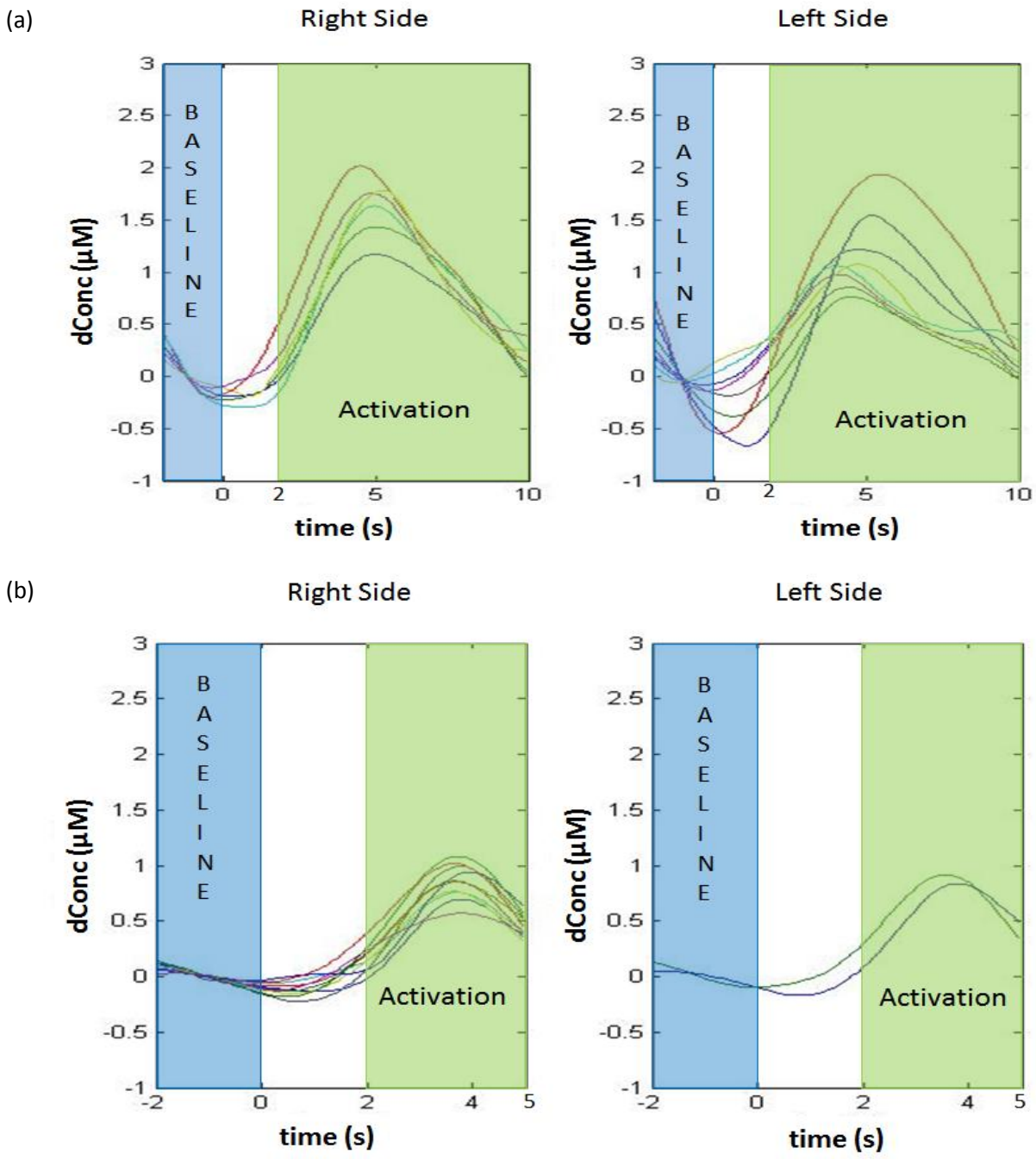


Figure 12: Block averaged channels in the region of interest for (a) DMS and (b) PVT

We consider -2 to 0 seconds as the baseline, marked in blue, and an activation, marked in green, from 2 to 10 seconds for DMS and 2 to 5 seconds for PVT. We also observe a dip between ~0 to 2 seconds which might be due to an overlap of the undershoot from the preceding blocks, hence we do not consider this data for our analysis with the control group.

The topographic images of the t-map for the channels in the ROI are as shown in figure 13.

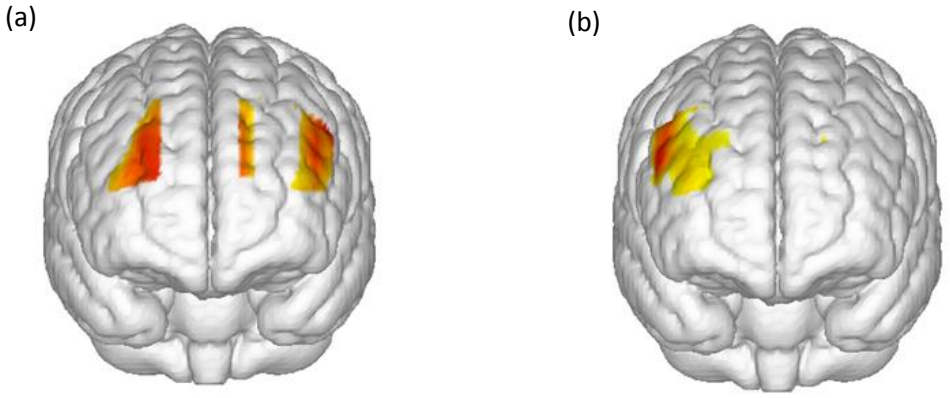
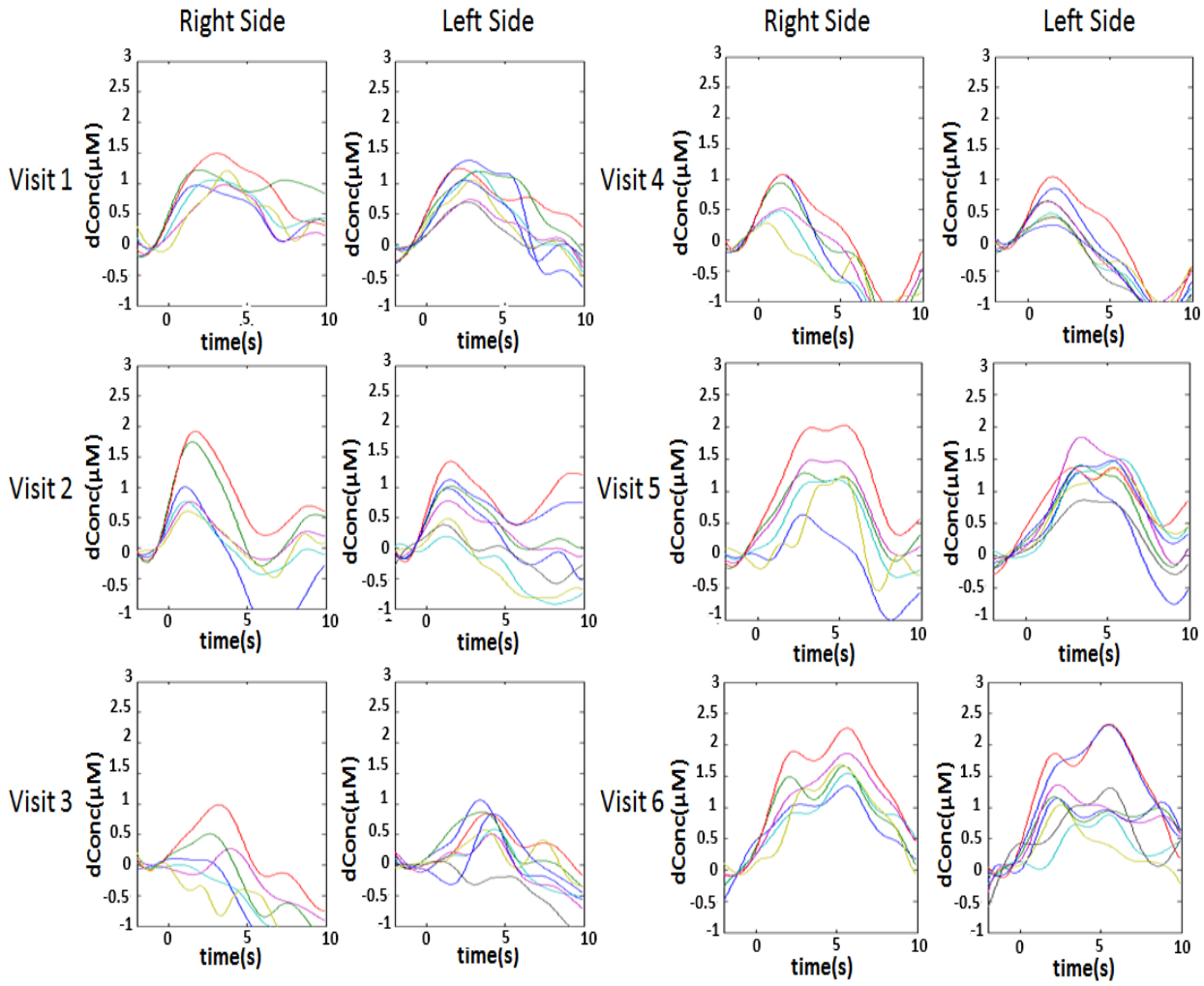


Figure 13: Topographic images of the t-map of significant channels for (a) DMS and (b) PVT

3.2.2 PTSD

The channels in the ROI for the PTSD subjects for pre-treatment and post-treatment over six treatment sessions are as shown in figure 14 and 15.

(a)


Right Side
Left Side
Right Side
Left Side
Visit 1
Visit 2
Visit 3
Visit 4
Visit 5
Visit 6
dConc(μM)
time(s)

(b)

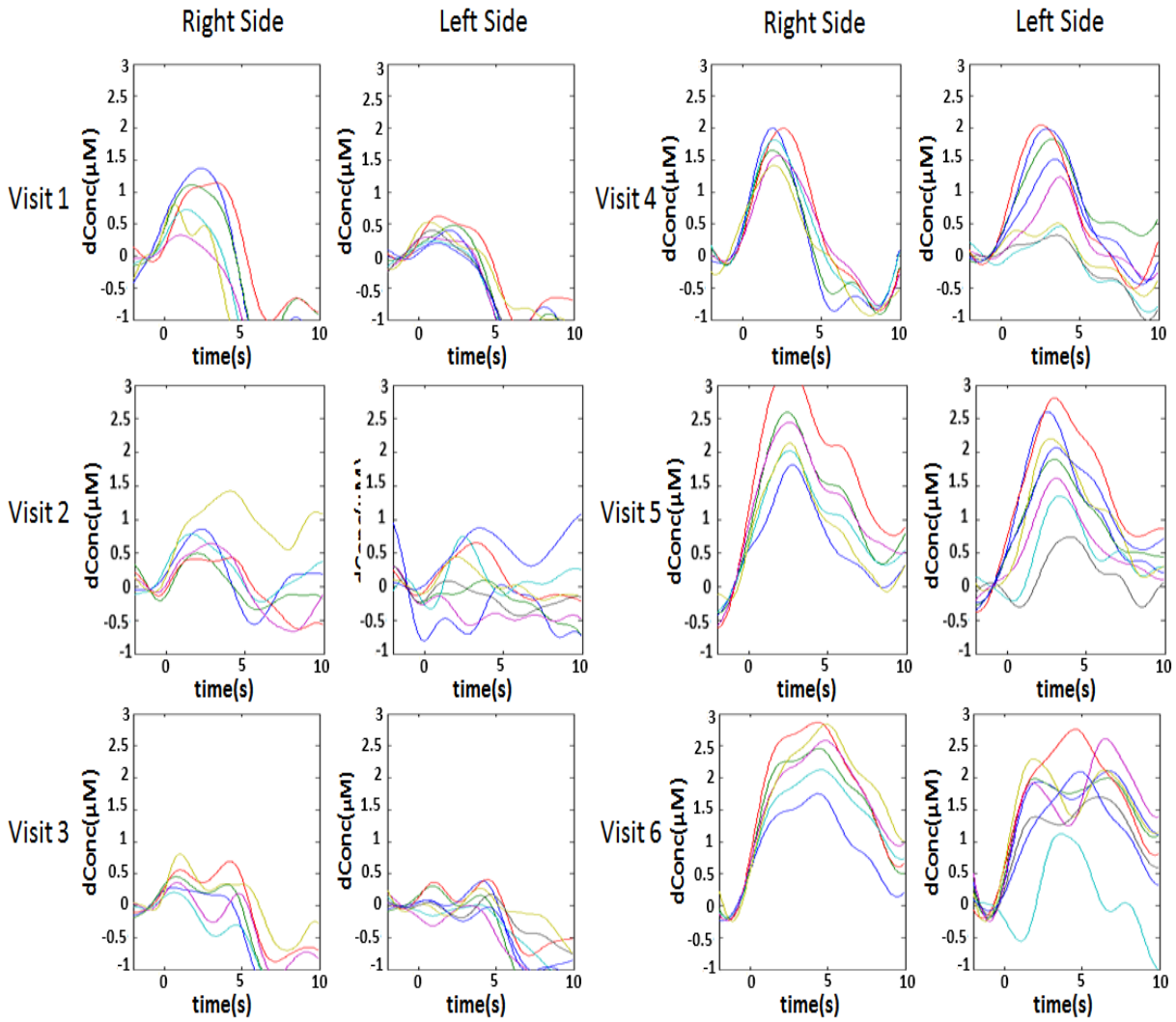
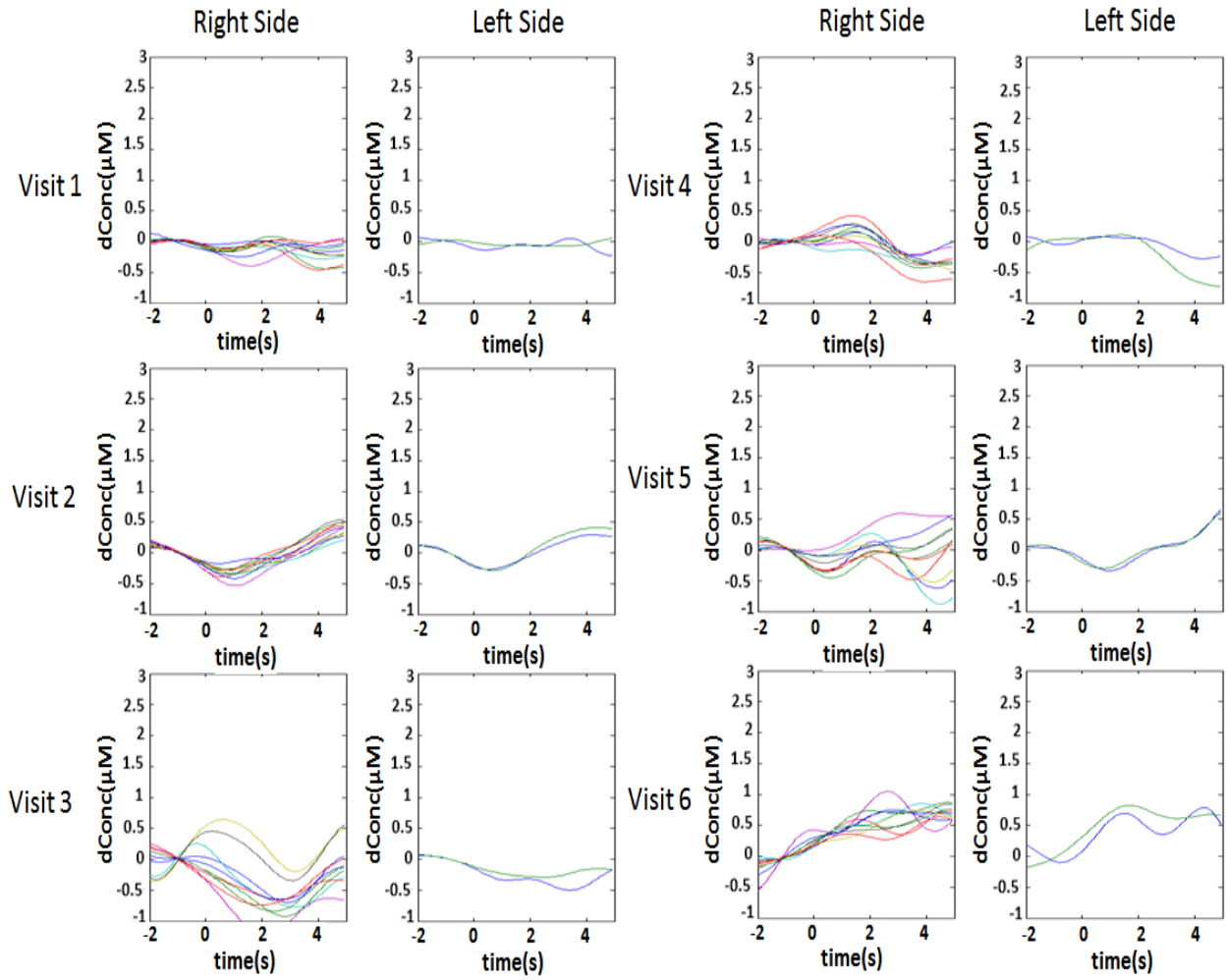


Figure 14: Plot of block averaged channels in ROI over 6 treatments for (a) Pre-Treatment and (b) Post-Treatment for DMS

(a)


Right Side
Left Side
Right Side
Left Side
Visit 1
Visit 2
Visit 3
Visit 4
Visit 5
Visit 6
dConc(μM)
time(s)

(b)

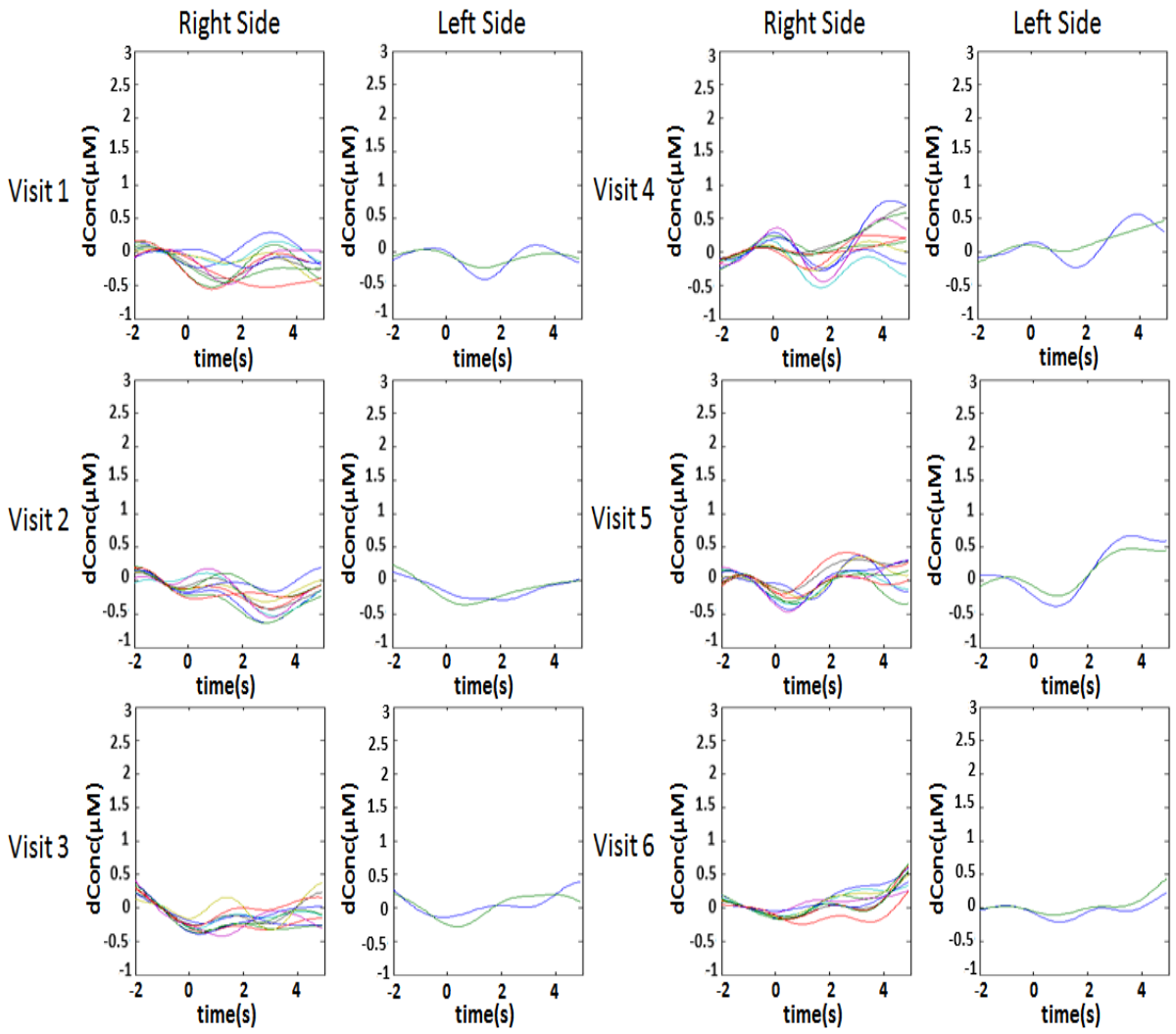


Figure 15: Plot of block averaged channels in ROI over 6 treatments for (a) Pre-Treatment and (b) Post-Treatment for PVT

From the plots in figure 14(a) for DMS, we can see that there is an overall increase in [HbO] changes from pre-LLLT 1 to pre-LLLT 6, however, there is a decrease in intensity in pre-LLLT 3 and 4 and then from pre-LLLT 5 again an increase in intensity is observed. We could also clearly observe two peaks in pre-LLLT 6 which represent two phases of memory namely, study and the recall phase. A similar increase in intensity is observed from post-LLLT 1 to post-LLLT 6, figure

14(b), with a decrease in intensity in post-LLLT 2 and 3 which are in agreement to the decrease seen in pre-LLLT 3 and 4. We could observe the two peaks, clearly, in post-LLLT 6 as well.

From the plots for PVT, from pre-LLLT 1 to pre-LLLT 6, figure 15(a), and post-LLLT 1 to post-LLLT 6, figure 15(b), we can observe that the [HbO] changes are low and there is no much change across six treatments.

Table 7 shows the result of paired sample two-tailed t-test for comparing pre-treatment and post-treatment data, changes in [HbO] integrated over time from 0 to 10 seconds for DMS and 0 to 5 seconds for PVT.

Table 7: Results (p-values) of comparison of pre and post treatment for (a) DMS and (b) PVT

(a)

	Right side	Left Side
Pre-LLLT1 Vs Pre-LLLT6	0.009	0.022
Post-LLLT 1 Vs Post-LLLT 6	0.002	0.009
Pre-LLLT 1 Vs Post-LLLT 6	0.001	0.002

(b)

	Right side	Left Side
Pre-LLLT 1 Vs Pre-LLLT 6	1.00E-08	0.093
Post-LLLT 1 Vs Post-LLLT 6	0.085	0.027
Pre-LLLT 1 Vs Post-LLLT 6	0.373	0.260

For DMS, the results show a significant difference ($p<0.05$) between pre-LLLT 1 and pre-LLLT 6 and also between post-LLLT 1 and post-LLLT 6. We also observed a significant difference ($p<0.05$) between pre-LLLT 1 and post-LLLT 6, which suggested that the treatment increased the hemodynamic response in PTSD after six treatments.

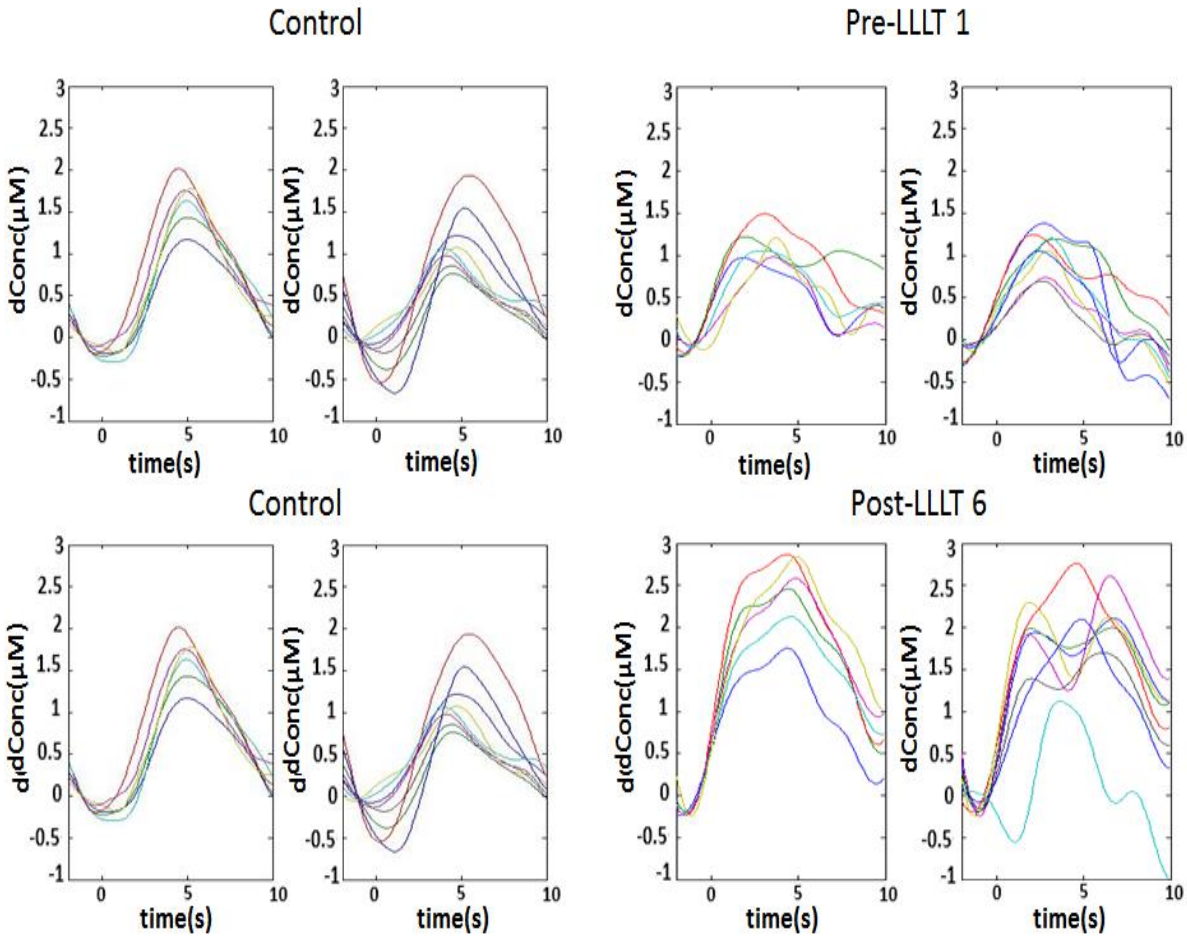
For PVT, we did see a significant difference between ($p<0.05$) between pre-LLLT 1 and pre-LLLT 6 but not between post-LLLT 1 and post-LLLT 6. We could not observe a significant

difference between pre-LLLT 1 and post-LLLT 6, suggesting that the treatment did not affect the hemodynamic responses occurring while performing PVT, over six treatment sessions.

3.2.3 Comparison between controls and PTSD

The plots of channels in the ROI for controls and pre-treatment 1 and post-treatment 6 are shown in figure 16.

(a)



(b)

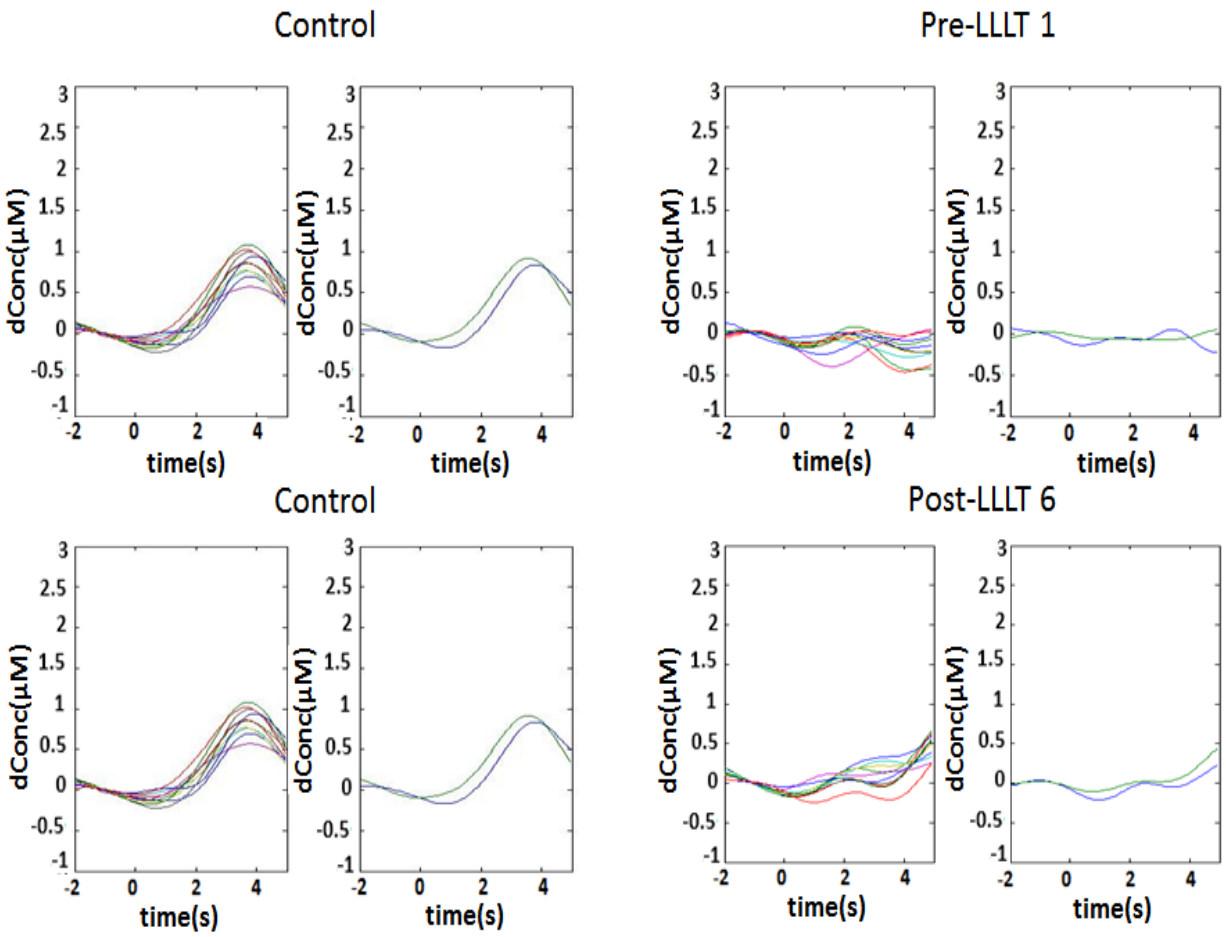


Figure 16: Plots of block averaged channels in ROI for controls, pre-LLLT 1 and post-LLLT 6 for (a) DMS and (b) PVT

For DMS, we can see that the [HbO] changes are higher in controls than in PTSD subjects before the first treatment i.e. pre-LLLT 1. However, the [HbO] changes are higher in PTSD after the last treatment i.e. post-LLLT 6 than in controls. This indicates that the treatment has had a beneficial effect on PTSD subjects after six treatment sessions. Table 8 (a) shows significant difference ($p<0.05$) between controls and PTSD subjects, for both pre-LLLT 1 and post-LLLT 6, confirming our observations from the plots.

For PVT, we can see that the [HbO] changes are higher in controls than PTSD subjects, for both pre-LLLT 1 and post-LLLT 6 indicating that the treatment did not have any effect on PTSD

subjects' hemodynamic responses occurring while performing PVT task. As the treatment did not have any effect on PTSD subjects we could observe a significant difference ($p<0.05$), as shown in Table 8(b), between controls and PTSD subjects for both pre-LLLT 1 and post-LLLT 6.

Table 8: Results (p-values) of comparison between controls and PTSD subjects (pre-LLLT 1 and post-LLLT 6) for (a) DMS and (b) PVT

(a)

	Right side	Left Side
Control Vs Pre-LLLT 1	0.031	0.047
Control Vs Post-LLLT 6	0.001	0.002

(b)

	Right side	Left Side
Control Vs Pre-LLLT 1	9.54E-09	0.006
Control Vs Post-LLLT 6	1.70E-08	0.012

Chapter 4

Discussion and Future Work

4.1 Discussion

There have been previous studies on the effect of LLLT on healthy controls. However, there have been very few studies on the effect of LLLT on veterans with PTSD. LLLT has been found to have beneficial effects on neurocognitive functions associated to pre-frontal cortex. In PTSD, the neurocognitive functions such as short-term memory and attention have been shown to be affected. This study is aimed at evaluating the effect of LLLT on veterans with PTSD focusing on the two cognitive domains, namely attention and short-term memory.

This study evaluates both behavioral and hemodynamic response in order to identify if the treatment had any beneficial effects on PTSD subjects.

The main findings of this study are as follows:

1. For PVT task, the behavioral scores showed no significant difference between controls and PTSD subjects and also for PTSD subjects across 6 treatments. The hemodynamic response showed significant difference between the controls and PTSD subjects, for both before the first treatment and after the sixth treatment, but not for PTSD subjects over six treatment sessions, indicating that the treatment did not have any effect on the PVT task.
2. For the DMS task, the behavioral scores showed significant difference between controls and PTSD subjects and also for PTSD subjects across 6 treatments. The hemodynamic response also showed a significant difference between the controls and the PTSD and also for PTSD over six treatment sessions. The results indicated that the treatment had a significant effect on the PTSD subjects for the task.

For the PVT task, the behavioral scores did not show any significant difference from the first to the last treatment. This could possibly be attributed to the protocol for the task. The difficulty level of the task was not sufficiently high to observe any difference across the six treatment sessions. As a result of which we could not evaluate the effect of the treatment on the attention domain more efficiently. The hemodynamic response occurring while performing the task also did not show much change across the six treatment sessions.

Basically there are two types of attention associated to two regions of the brain, namely pre-frontal cortex and parietal lobe. The pre-frontal cortex is mainly responsible for willful attention, for example, studying for a test or writing novel. The parietal lobe on other hand is responsible for automatic attention, for example, sudden attack of an animal or the scream of a child. A study by Miller et al had shown that when a stimulus pops up suddenly, parietal cortex directs our eyes towards the stimulus, however, when a person is looking for something, the pre-frontal cortex is the driving force of attention. PVT task involves both kinds of attention where in the person looks for the counter to start (willful attention) and also when they are instructed to stop the counter as soon as it appears (automatic attention) and hence involves both the regions of brain mentioned earlier [37]. Hence, a possible reason for not observing any change on the attention domain for the PTSD subjects after six treatment sessions could be attributed to the laser stimulation site, in our current study we focused only on laser stimulation of pre-frontal cortex and not on the parietal lobe.

DMS task is a test for short-term memory, requiring the subject to remember visuospatial information (a randomly generated pattern in the current study). It has been shown that the dorsolateral pre-frontal cortex plays a critical role in visuospatial memory [38]. As we focus

the laser stimulation on the prefrontal cortex, the treatment showed to have beneficial effects on short-term memory of PTSD subjects.

Another important feature that we observed was that in the beginning of each trial, of DMS task, hemodynamic response increases slowly in controls than in PTSD subjects, i.e. in PTSD subjects, hemodynamic response increases immediately when a trial starts resulting in shorter time-to-peak in PTSD subjects than in controls. Also, the hemodynamic response remains high for a longer duration in PTSD subjects than in controls, during the trial. This might be attributed to, controls performing the task with less efforts than PTSD subjects. As PTSD subjects perform the task with more efforts, their hemodynamic response reaches peak much faster than the controls, who perform the task with minimal efforts.

4.2 Limitations

The current study had a few limitations as mentioned below: -

1. The study was conducted with a small sample size of 4 PTSD subjects, as a result of which, it was difficult to clearly determine the beneficial effects of the treatment, statistically, for both behavioral and hemodynamic response.
2. The age of 8 healthy controls used for this study ranged from 24 to 40 years among them, 7 controls were in the age range of 22 to 26 years and only 1 subject was in the higher end of the range. However, the age of the 4 veterans with PTSD ranged from 25 to 45 years. Hence, for this study, the controls were not age matched with PTSD subjects, which could have led to a bias in the statistical analysis, due to confounding.
3. Due to the low level of difficulty of PVT task, the behavioral scores of PTSD subjects did not show a significant difference across six treatments as well as between the PTSD subjects and controls.

4. Although, attention is a function associated to both pre-frontal cortex and parietal lobe, the latter brain area plays a major role in attention. An improvement in the performance of PTSD subjects on the attention task could not be observed in this study as it was mainly focused on laser stimulation of pre-frontal cortex.

4.3 Future Work

Based on the findings from the current study the future work could be directed towards the following:

1. An increase in sample size of PTSD subjects could increase the chance of finding more conclusive results from the data and could also ease the statistical analysis.
2. We could select a few more aged healthy controls, in order to have an age matching with the PTSD subjects to avoid any bias in the statistical analysis.
3. As the difficulty level of PVT task is not sufficiently high to evaluate the effect of treatment on the attention domain efficiently, a change in protocol of the study, for example using a neurocognitive task testing attention domain with higher difficulty level, could be used.
4. Other brain regions associated to attention, for example parietal lobe, could be of focus for laser stimulation in future studies to evaluate the effect of treatment on hemodynamic changes occurring while performing task (testing attention) more efficiently.
5. As this study is based on event-related paradigm, general linear model (GLM) analysis could be more efficient and accurate in determining the hemodynamic changes.

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