HEMODYNAMIC CORRELATES OF MENTAL ARITHMETIC TASK IN MIGRAINE

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This work is dedicated to my parents.

ABSTRACT

HEMODYNAMIC CORRELATES OF MENTAL ARITHMETIC TASK IN MIGRAINE

Investigating the relationship between the hemodynamic changes and cognitive activity (known as the neurovascular coupling) provides a basis of the underlying physiology of the brain energy mechanisms. The aim of this study was to investigate the differences in the hemodynamic response caused by the mental arithmetic (MA) task between migraineurs and healthy subjects by using functional near infrared spectroscopy (fNIRS). 16 healthy subjects (5 male, 11 female) and 16 migraine patients (4 male, 12 female) participated in the study. Subjects were asked to perform mental subtraction and answer verbally to 3 sets of questions with increasing complexity. Performance, work load, FNIRS data and laterality (LI) index were analyzed. The difference in the oxyhemoglobin levels across different complexity levels were calculated. As the MA task got harder, work load increased, performance decreased and the change in [HbO_2] increased for both groups but showing a lower incremental in oxy-Hb concentration in migraine patients for varying complexity levels. Control group showed a right dominant PFC activity, whereas migraine patients showed a left dominant PFC actvity. Our results support the hypothesis that migraine is a neurovascular coupling dysfunction causing unregulated activation in PFC than controls.

Keywords*: fNIRS, migraine, mental arithmetic, laterality index, prefrontal cortex.*

ÖZET

MİGRENDE ZİHİNSEL ARİTMETİĞİN HEMODİNAMİK İLİŞKİLENDİRİLMESİ

Kognitif aktiviteler ve hemodinamik değişiklikler arasındaki ilişkiyi (nörovasküler kuplajı) incelemek, beyin enerji mekanizmalarının altında yatan patofizyoloji hakkında bir zemin oluşturmaktadır. Bu çalışmanın amacı migrenli hastalar ve sağlıklı insanlar arasında, bir mental aritmetik (MA) testi sırasında prefrontal kortekste (PFK) oluşan hemodinamik tepkilerdeki değişikliklerin işlevsel yakın kızıl-altı spektroskopisi (iYKAS) yöntemiyle incelenmesidir. Çalışmaya 16 sağlıklı (5 erkek, 11 kadın), 16 migren hastası (4 erkek, 12 kadın) katılmıştır. Kişilerden zihinden çıkarma işlemi yapmaları ve gittikçe zorluğu artan, 3 setten oluşan soruları sözlü bir şekilde cevaplamaları istenmiştir. Performans, iş yükü, İYKAS datası ve lateralite indeksi (LI) analizleri yapılmıştır. Değişik zorluk seviyelerinde Prefrontal kortekste oluşan oksihemoglobin değişiklikleri hesaplanmış. MA testi zorlaştıkça, değişken zorluk seviyeleri için, iş yükü artmış, performans düşmüş, ve migren hastalarında daha az olmak üzere HbO_2 konsantrasyonunda artış gözlenmiştir. Kontrol grubunda sağ tarafta baskın PFK aktivitesi gözlenirken, migrenli hastalarda sol tarafın baskın olduğu gözlenmiştir. Sonuçlarımız, migrenin PFK'da sağlıklı insanlardakine göre düzenlenmemiş aktivasyona sebep olan bir nörovasküler kupling bozukluğu olduğunu desteklemektedir.

***Anahtar sözcükler**: fNIRS, migren, mental aritmetik, lateralite indeksi, prefrontal korteks.*

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LIST OF ABBREVIATIONS

ANS	Autonomic nervous system
CBF	Cerebral blood flow
rCBF	Regional cerebral blood flow
CSD	Cortical spreading depression
EEG	Electroencephalography
FHM	Familial hemiplegic migraine
fNIRS	Functional near infrared spectroscopy
fMRI	Functional magnetic resonance imaging
Hb	Deoxy-haemoglobin
HbO_2	Oxy-haemoglobin
HR	Heart rate
LI	Laterality index
MA	Mental arithmetic
MEG	Magnetoencephalography
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NIRS	Near infrared spectroscopy
NVC	Neurovascular coupling
PET	Positron Emission Tomography
PFC	Prefrontal cortex
SPECT	Single Photon Emission Computed Tomography
TSD	Transcranial Doppler sonography
WL	Work Load
WM	Working memory

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1. INTRODUCTION

1.1 Motivation and Objectives

The cerebral vessels react by vasodilatation to cognitively-induced neuronal activation leading to changes in blood flow. This phenomenon is known as the neurovascular coupling (NVC). Researchers have hypothesized that the underlying pathophysiology of many brain disorders (i.e. psychiatric, neurological, vascular disorders) could be related to the impairement in NVC mechanisms. Many cognition-induced tasks have been developed that result in quantifiable blood flow changes to highlight the NVC disorders. Among these is the Mental Arithmetic test (MA task).

Mental arithmetic as a mental stressor is shown to have an activation pattern in prefrontal cortex (PFC) with respect to the according to stress and the mental calculation. Mental calculation is known to exploit working memory. Working memory stores and manipulates information for a short period of time in order to process other complex cognitive tasks. This cognitive process results in a hemodynamic response in the PFC blood vessels via changes in concentration of oxygenated Hemoglobin.

The exact mechanism underlying the pathophysiology of migraine is still a subject of debate with many controversial points of views. Research on causes of migraine has centered on biochemical imbalances, neurotransmitter dysfunction, vascular disorder and autonomic dysfunction. Recent studies have focused on the unregulated vascular responses of migraine patients to different types of mental and physical stress inducers. Hence recent hypotheses on migraine pathophysiology have been revolving around the NVC hypothesis of migraine.

In this study, the hemodynamic response during mental arithmetic task was evaluated in both healthy subjects and migraine patients and these responses were compared among these two groups. Our specific hypothesis was that “The hemodynamic response increases with the increasing working memory load in all subjects and this

increase would be somewhat impaired in migraine patients according to the neurovascular coupling dysfunctioning hypothesis of migraine."

The aim of this study is to evaluate the differences of activation and oxygenation of prefrontal cortex between healthy subject and migraineurs and to improve our understanding of the neurovascular coupling disorder of migraine during a cognitive task by using Near-Infrared Spectroscopy (NIRS).

1.2 Contribution of the Thesis

The major contribution of this thesis work is examining the changes in cerebrovascular changes in migraine patients and control group due to the effect of a mental activity and comparing the amount of changes in blood oxygenation with healthy subjects to show the NVC disorder and have a better understanding of the physiological changes in the brain during mental work. An experimental protocol is designed;

1. to generate a higher activity in the prefrontal cortex by using a cognitive task, which is composed of three difficulty levels,
2. to measure the activation differences between healthy and diseased (migraine) subjects.

To our knowledge, this study will be the first in literature to apply MA task to migraine patients with functional near infrared spectroscopy.

1.3 Outline of the Thesis

Chapter 1 introduces the thesis. Brief information about the functional neuroimaging is given in Chapter 2. Chapter 3 explains the MA task optical imaging techniques to measure mental activity. Chapter 4 gives detailed information about the pathophysiology of migraine and functional neuroimaging studies. Chapter 5 provides a broad explanation on the design of experimental protocol and data analysis procedure. Results of the experiments, comparison with previous studies and recommendations for future work are given in Chapter 6. Finally, Chapter 7 mentions concluding remarks for the study.

2. FUNCTIONAL NEUROIMAGING

An important field in neuroscience is the imaging of the brain in order to find out the biological basis of emotion, cognition and consciousness. As this vast network is made of billions of neurons, the brain activity is generated and transferred through these neurons. This information is shared with neighboring neurons resulting in a local function or carried to different parts of the brain, resulting with a global function [1]. To visualize and measure the neuronal activity functional neuroimaging methods are used [1, 2]. Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT), functional Magnetic Resonance Imaging (fMRI), Electroencephalography (EEG), Magnetoencephalography (MEG) and Near Infrared Spectroscopic imaging (NIRS) are the basic functional imaging modalities. These methods can measure the electromagnetic signals or physiologic and metabolic changes as a result of neuronal activity.

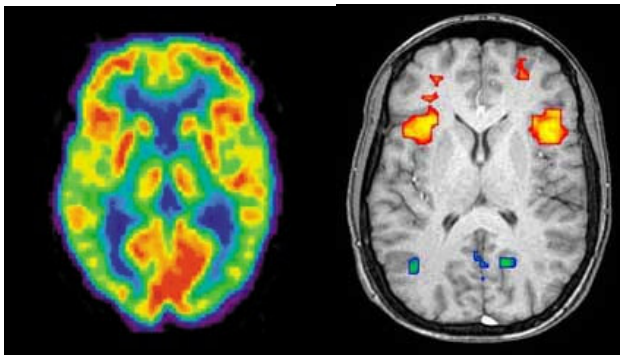

Figure 2.1 A PET scan from a study done investigating Alzheimer's (on the left) and an axial MRI scan at the level of the basal ganglia (on the right) [3].

Functional neuroimaging techniques are actually based on finding the effects of the brain functions on different brain areas [1]. PET, fMRI and NIRS methods are based on observing the regional cerebral blood flow changes in response to a neuronal activity, whereas EEG and MEG are based on observing the electromagnetic signals produced

during brain activity. First three methods also give spatial and temporal information about the neuronal work as well as the simultaneous recordings performed by EEG and MEG [2].

2.1 Optical Imaging

One type of the functional neuroimaging technique is the optical imaging, which is based on the interaction between the light and the brain tissue that provides functional information of the interested brain region [4]. Light tissue interactions can be absorption, florescence, phosphorescence, scattering or Doppler shift. Each interaction and each method such as; NIRS, florescence measurements by using potential sensitive dyes or calcium sensitive dyes and laser Doppler flowmetry give different information about the neuronal changes [4, 5].

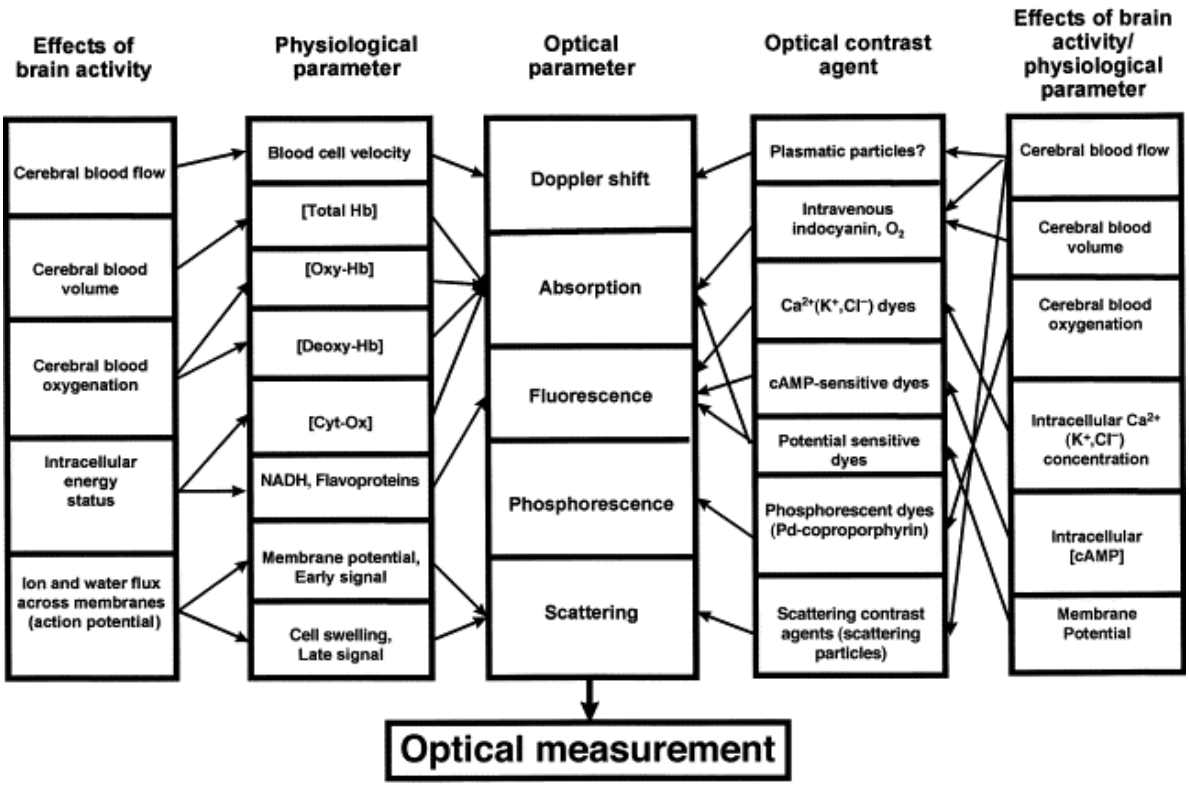

Figure 2.2 Evaluation of brain activity by measuring optical parameters [5].

Optical imaging is non-invasive and gives information about the concentration of a specific chemical in the brain [4]. This method has many advantages when compared with

other imaging techniques: optical imaging devices are cheaper, portable, and safer and has a better signal-to-noise ratio (NIRS) [6].

Light absorption property of the optical imaging measures the endogenous substances such as oxygenated Hemoglobin (oxy-Hb), deoxygenated Hemoglobin (deoxy-Hb) and cytochrome-C-oxidase (Cyt-Ox) by using different and characteristic absorption patterns of visible and near infrared light [4].

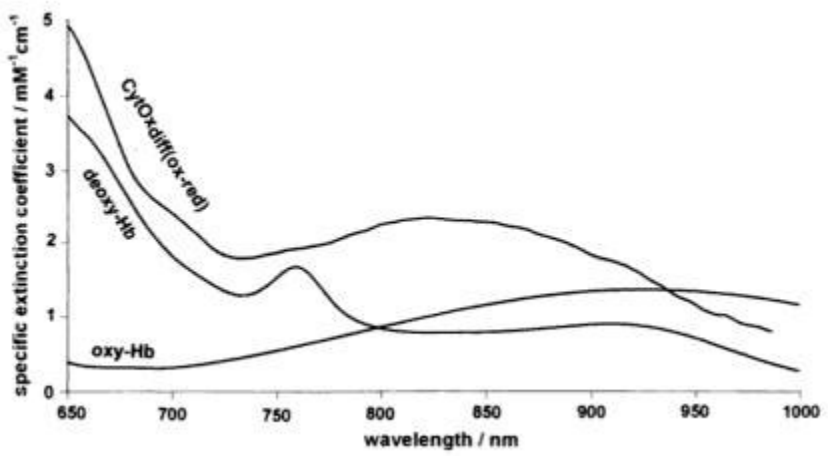

Figure 2.3 Graph showing the absorption spectra of oxy-Hb, deoxy-Hb and $CytOx_{diff}$ (difference between oxy-Hb and deoxy-Hb form) [4].

Some events can affect the hemoglobin oxygenation in the interested brain region according to the neuronal activity. Therefore, changes in oxygen consumption, blood volume and cerebral blood flow (CBF) are main concepts of interest. Existing studies showed that during functional brain activation situations, oxy-Hb concentration increases whereas deoxy-Hb decreases [4].

2.2 Functional Neuroimaging In Cognitive Neuroscience

Cognitive neuroscience is a multidisciplinary combination of neuroscience and cognitive psychology in science aiming to find the reaction of the brain to a specific mental

stimulator. In order to localize and measure the brain activity caused by this mental activator, functional imaging techniques have been used to lighten the relationship between brain and behavior. They showed that specific brain regions are responsible for specific mental activities [7]. These functions, which can be performed by human and other primates, are maintained by working memory (WM) which is the capacity of understanding complex cognitive processes by holding and storing the information for a short period of time and operate the data. The activation of the working memory was imaged with different modalities such as PET and fMRI, and they all showed activations in different regions, but commonly in PFC [8-14]. These studies all confirm the fact that brain needs energy to perform cognitive activity due to a mental activator, and the supply is the oxygen and glucose which are maintained by continuous regulation of the blood flow. Hence, the changes in the blood content within a region provides an idea about the amount of the energy needed, and the amount of the activity performed [15, 16, 17].

The relationship between the neuronal activity and the blood content variations is called “neurovascular coupling” as the changes in the blood are controlled by the vasoactivity of the vessels [18]. This coupling can be imaged by PET monitoring the increase in cerebral blood flow, in glucose utilization and oxygen consumption; by fMRI monitoring the signals generated by the degree of blood oxygenation and by magnetic resonance spectroscopy (MRS) visualizing the changes in the local concentration of certain metabolic intermediates, such as lactate and glucose, showing neuronal activity [16]. PET is based on introducing a molecule that is radioactively labeled with an unstable radionuclide, into the metabolism and see the localization of this molecule according to the activity. On the other hand, fMRI is based on detection of magnetic signals detected in an activated brain region according to the amount of oxygenation. Altering MRI signals can be detected depending on the oxy-Hb/deoxy-Hb ratio in the interested brain area [18].

According to the NVC hypothesis, it is thought that astrocytes have an important pivotal role in the distribution of energy metabolites produced during the energy circulation to the neurons. Astrocytes, known as satellite cells, have multiple fine processes that some of them are in close apposition to capillary walls. Astrocytic end-feet cover the whole surface of intraparenchymal capillaries. Astrocytes become a likely site of prevalent glucose uptake by forming the first cellular barrier that glucose entering the brain parenchyma encounters. As well as this, GLUT1 of glucose transporters are expressed on

astrocytic end-feet. There are also other processes that take place through synaptic contacts which have receptors for different types of neurotransmitters and reuptake regions as well. Previous quantitative morphometric studies support that astrocytes are polarized cells, with one process contacting a mesodermal cell (frequently an endothelial cell of the capillary) and a multitude of process that are intertwined within the neuropil ensheathing synaptic contacts. These processes make up approximately 80% of an astrocyte membrane surface and do not include organelles. By this organization of processes, astrocytes can both recognize the changes in the synaptic activity and be able to couple it with brain energy metabolism [16, 17].

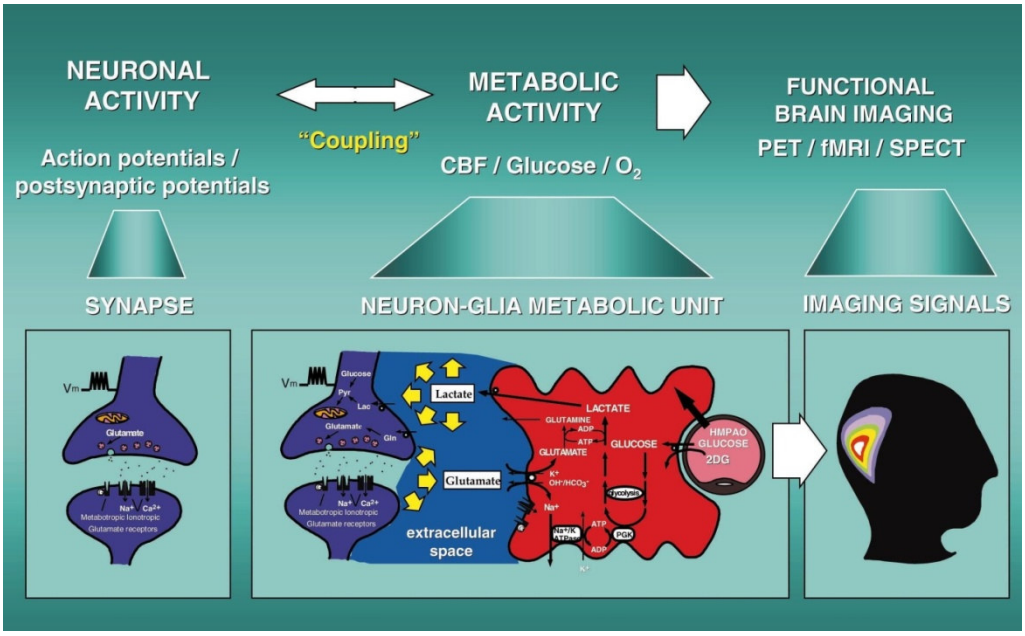

Figure 2.4 Scheme showing glutamatergic synapse (left panel), neurovascular coupling activity (middle panel) and functional neuroimaging (right panel) [16].

It is known that glutamate is an important neurotransmitter in neuronal activity. Therefore when a neuronal signal reaches the presynaptic neuron, that neuron secretes glutamate. At glutamatergic synapses, secreted glutamate acts at a specific receptor and depolarizes postsynaptic neurons. Astrocytes end this action by an efficient glutamate uptake system. 1 glutamate molecule is taken in with 3 Na^+ ions, causing an increase in the

intra-astrocytic concentration of Na^+. This activates the astrocyte Na^+, K^+-ATPase and it stimulates the use of 1 molecule of glucose and production of 3 molecules of lactate, which is called glycolysis. During this process 2 molecules of ATP is produced. One ATP is used to turn on the pump, while the other is used to convert glutamate to glutamine by glutamine synthase. When lactate is released, it is taken up by post or presynaptic neuron to produce energy. Lactate in astrocytes and glutamate in neurons can also be produced from the glucose taken from the blood vessels. On the other hand, metabolic intermediates like Alanin, Glutamine, Glutamate, and α-ketoglutarate can also be secreted and taken in between astrocytes and neurons [16, 17, 18].

Functional imaging techniques such as PET and MRS also support the NVC [17]. However there is another case where uncoupling may also be present.

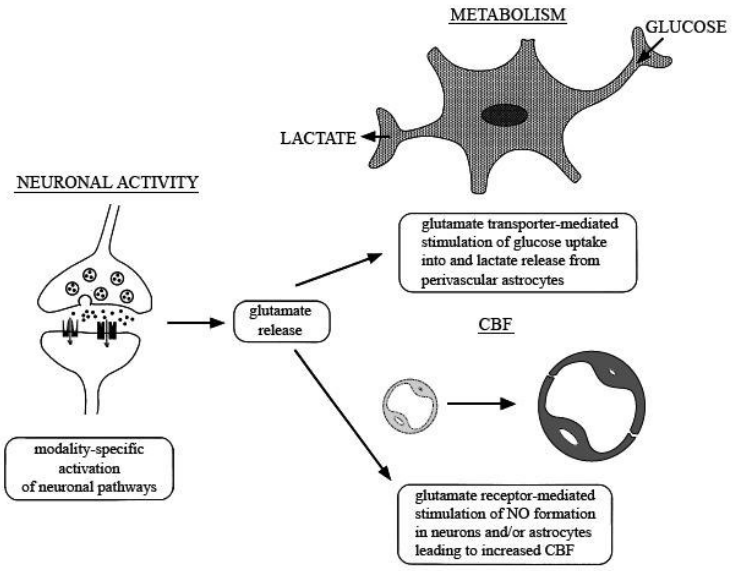

Figure 2.5 In-parallel regulation of activity-linked increase in metabolism and CBF [17].

Metabolism and CBF can be uncoupled under some selective pharmacological manipulations or particular pathophysiological conditions. This can be seen and named as in-parallel regulation of activity linked increase in CBF and metabolism. As glutamate is secreted from active synapses, receptor mediated NO is formed in neurons. This may assist

to the increase of activity-dependent CBF. At the same time, glucose use and production of lactate is seen in astrocytes as a result of glutamate reuptake [17].

Screening the changes in the oxygenation of the brain and other metabolites gives information about the brain work performed by showing increases or decreases in the signals or outputs of the imaging system [15].

2.3 Optical Neuroimaging in Prefrontal Cortex

Prefrontal Cortex (PFC) is known to be responsible of planning complex cognitivebehaviors, expression of personality, making decisions and mediating socially correct behaviors [19]. The most important and observed responsibility of PFC is its executive functions, which include moderating conflicting thoughts, making choices between right and wrong, good and bad, same and different, predicting future events, and controlling social acts such as suppressing emotional or sexual needs [19, 20].

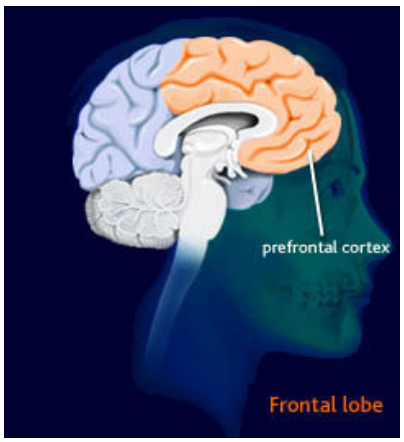

Figure 2.6 Prefrontal cortex of a human brain [19].

The role of PFC in cognitive activities made it a target for researchers to image the activity and possible changes in this part of the brain. Apart from other functional

neuroimaging methods, optical imaging modalities such as NIRS have become more advantageous to be used in imaging PFC, since they are inexpensive, non-invasive, can be used repeatedly without any harm or adverse event and can be used both on children and adults [21, 22]. Therefore, many optical imaging studies have been done in order to find out emotional, cognitive, psychology-related effects on the PFC [23-28].

3. Mental Arithmetic Task

Mental arithmetic is a simple calculation such as addition or subtraction which involves attention, working memory and executive functions to be performed [29]. Functional neuroimaging studies employing the MA test have used the positron emission tomography (PET), functional magnetic resonance tomography (fMRI) techniques [29, 30, 31]. According to the literature, it is shown that patients with lesions in the inferior parietal lobe fail to perform simple number calculations, the angular and supramarginal gyri are critically involved brain areas for mental arithmetic, recent studies involving healthy subjects and using hemodynamic neuroimaging methods fMRI and PET have supported the literature on lesions by showing that inferior parietal and prefrontal cortical areas are activated during mental arithmetic tasks [29]. Moreover, previous studies have shown that mental arithmetic test causes activation in the lateral and ventral prefrontal cortex, posterior parietal lobe, cerebellum and the subcortical regions including the caudate nucleus [30]. A study done by Inoue et al. (1993) by using EEG showed that the left temporo-centro-parietal activation is specific to calculation processing, and the frontal information flow is related to the active performance of mental arithmetic [31]. Studies performed on brain damaged patients showed that the lesions of the PFC cause deficits/impairments in working memory and mental calculation [32, 33]. By virtue of this information, MA test may be an effective method to investigate the activation changes in PFC.

Existing studies showed that increasing workload by increasing the difficulty of the task caused the activation of the PFC [34, 35, 36, 37].

Mental arithmetic tasks are also used as stressor tests and heart rate changes and cerebral blood volume changes are observed during these tests [27, 28].

3.1 Optical Imaging Findings on Mental Arithmetic Task

Usage of NIRS technology shows activation in prefrontal cortex, supporting the previous findings done by other functional neuroimaging modalities and also increase in heart rate [27, 28]. Increase in the concentration of oxy-Hb and total-oxy with a decrease in the concentration of deoxy-Hb and regional CBF increases were also seen bilaterally or laterally in PFC [27, 28, 38].

4. Migraine Pathophysiology

Migraine is a neurological disorder that affects human a higher prevalence in women (15%) than in men (6%). Migraine is explained by an intense and throbbing unilateral headache related with anorexia, nausea, vomiting, photophobia, phonophobia and/or diarrhea (common migraine). In some cases, headache may be preceded by a focal neurological phenomenon (“aura”) and followed by headache (classical migraine); this aura consists of certain motor (weakness or paralysis) and/or visual (scintillating scotoma) focal neurological symptoms [39, 40].

Migraine was assumed to be caused by extracranial arterial vasodilation and extracranial neurogenic inflammation as pathogenic mechanisms [41, 42]. The vasogenic theory tells that intracranial vasoconstriction was responsible for the migraine aura and migraine pain is a result of a rebound dilation and distention of cranial vessels and activation of pervascular nociceptive axons [43]. The neurological theory of migraine proposed that migraine resulted from abnormal firing in brain neurons. Cortical spreading depression, one facet of the neurological theory, could explain the aura of migraine. The neurogenic dural inflammation theory of migraine supposed that the dural membrane surrounding the brain became inflamed and hypersensitive due to release of neuropeptides

from primary trigeminal sensory nerve terminals. Substance P, calcitonin gene related peptide and nitric oxide are all thought to play a role in the dural inflammatory cascade [40, 44, 45].

It is found that in migraine sufferers, during and between migraine attacks, small blood cells, or platelets, do not behave normally. The purpose of the blood platelets is to aggregate, or to clump together, to form blood clots. In migraine sufferers, there is a significant increase in the spontaneous aggregation, or clumping, of blood platelets. This causes a reduction in the release of a brain chemical known as serotonin. Serotonin aids in the chemical transfer of information from one cell to another. More importantly for migraine sufferers, serotonin plays a major role in the relaxation and the constriction of blood vessels. All of the serotonin in the blood is stored in the blood platelets. It is released by platelet aggregation [46].

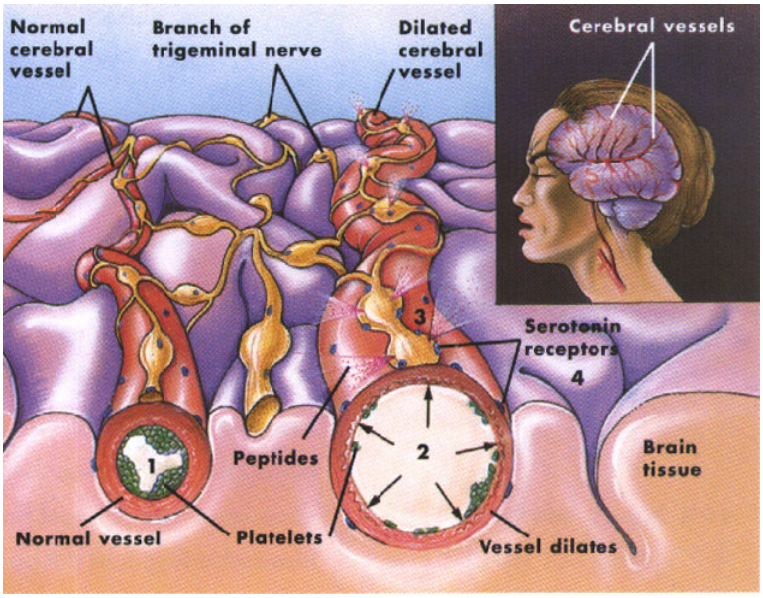

Figure 4.1 (1) Blood vessels have an intrinsic tone under normal conditions. (2) During a migraine attack, the blood vessels dilate. This "stretching" of the blood vessel walls may produce migraine symptoms. (3) Migraine pain also may stem from the release of peptides from the trigeminal nerve terminals, which project to the blood vessels. The peptides may alter pain thresholds. (4) Many researchers believe that sumatriptan and other related antimigraine drugs under investigation counter one or both of these migraine contributors through their actions on specific serotonin receptors [44].

4.1 Cerebrovascular Autoregulation Disorder

Vasodilation is not enough to explain the local swelling and tenderness of the head that generally accompanies the migraine. With respect to the neurovascular theory, it is proposed that vascular change is secondary to neural activation. According to this theory, there is abnormal neuronal excitability in the cerebral cortex, possibly because of reduced levels of magnesium and increased levels of calcium and glutamate, producing susceptibility to CSD in migraineurs, and peripheral sensitization of the trigeminal vascular system. Pain is generated from the dura and blood vessels and is enhanced by neurogenic plasma protein extravasations, which is mediated by neuropeptides. As well as this, central neural processing occurs through mechanisms of pain modulation and central sensitization [45, 46].

Migraine is thought to be the result of an uncoupling of the neuronal activity and the hemodynamic response. Existing hypothesis suggest that excess amount of glutamate is present in the synaptic cleft caused by the effected uptake of glutamate in the presynaptic astrocyte takes place as a result of the increased glutamate secretion in the presynaptic cleft according to a mutation of the P/Q type Ca^{++} channel or a Na-K ATPase mutation in patients suffering from familial hemiplegic migraine (FHM). These mutations explain the CSD by the excessive excitability of neurons of these patients. Further more, the Na/K ATPase causes lactate production by anaerobic glucose metabolism and neurons use lactate as energy source because of its location in the metabolic pathway adjusting the uptake of glucose uptake from the blood with respect to glutamate levels.

4.2 Functional Neuroimaging In Migraine

Different neuroimaging techniques have been used to visualize and observe the cerebral hemodynamic response such as; SPECT, PET, fMRI, MRS and transcranial Doppler Sonography (TSD). These results show an increase in the blood flow velocity during interictal period and increased blood flow during migraine attacks. However most

of them examine the middle cerebral artery and observations demonstrated that no significant changes occur in MCA during migraine or migraine-free periods. Migraine with and without aura also showed contrasting results in changes in rCBF [43, 48, 49, 50, 51, 52].

5. EXPERIMENTAL PROCEDURES

5.1 Subjects

5 male and 11 female healthy subjects (aged 20-40 years; mean 24+- 4.15 years) as the control group, who are non-headache sufferers; 4 male and 12 female migraine patients (aged 20-40 years; mean 23.94+- 3.45 years) participated in the present study. All of the subjects included in this experiment are college educated. Both of the two groups were informed personally about the procedure. In order to eliminate the environmental noises, subjects were seated in a comfortable chair in a dimmed room. Subjects in their menstrual period, those with known cardiovascular, neurological or psychiatric disorders, and those experienced a migraine attack in 3 days before the experiment and those using an acute attack medication and analgesic intake 24 hour priority were excluded from participating.

5.2 Task Protocol

The MA task consisted of three sets of questions with increasing complexity. In the first set, the subjects were asked to subtract serially a 2-digit number from a 2-digit number (e.g. 33-18), while in the second set a 2 digit number from a 3-digit number (e.g. 457-69) and finally for the third set a 2 digit number from a 4 digit number (e.g. 1406-45) as quickly as possible. The data collection started with a one minute of baseline. Each set lasted a minute and following each set participants were allowed to rest for a minute during which we continued recording. The task protocol was approved by the Human Research Ethics Committee of Boğaziçi University.

5.3 fNIRS Data Acquisition

fNIRS data were collected by the NIROXCOPE 301, which was developed at the Biophotonics Laboratory at Boğaziçi University. The NIROXCOPE 301 contains 4 LED sources and 16 detectors. Detectors and sources are placed symmetrically in a rectangular

probe with 2.5 cm spacing between each source detector pair and this probe is placed on the forehead of the subjects with the help of rubber bands as shown in Figure 5.1. Data collection started with the resting state and ended with resting state in order to observe the baseline measurements.

Figure 5.1 A typical set up for the MA task.

NIROXCOPE 301 houses multi-wavelength LEDs, which emit light at 2 different wavelengths (730 and 850 nm). Changes in the concentrations of oxy-Hb and deoxy-Hb are calculated by the modified Beer-Lambert Law from intensity of light that returned to the detector.

5.4 Performance Analysis

The behavioral performance measure was taken as the percentage of wrong answers at each level of complexity. For each subject, wrong answer percentage was calculated by taking the ratio of the number of wrong answers to the total number of answered questions. If a subject made an error on 80% of his/her responses, then their data was discarded and not included in inferential analyses. In order to compare whether there was an effect of complexity on the percentage of wrong errors, the data were submitted to a repeated measures ANOVA. The performances for men and women were one way ANOVA tested

to see if there is a gender difference among the subjects and it was confirmed that the data had no significant sex difference ($p>0.05$)

5.5 Data analysis

A MATLAB based program was developed and used for analysis of fNIRS data. Figure 5.2 displays an example of fNIRS signal composed of Hb, HbO_2, Blood Volume and OXY concentrations during the MA task.

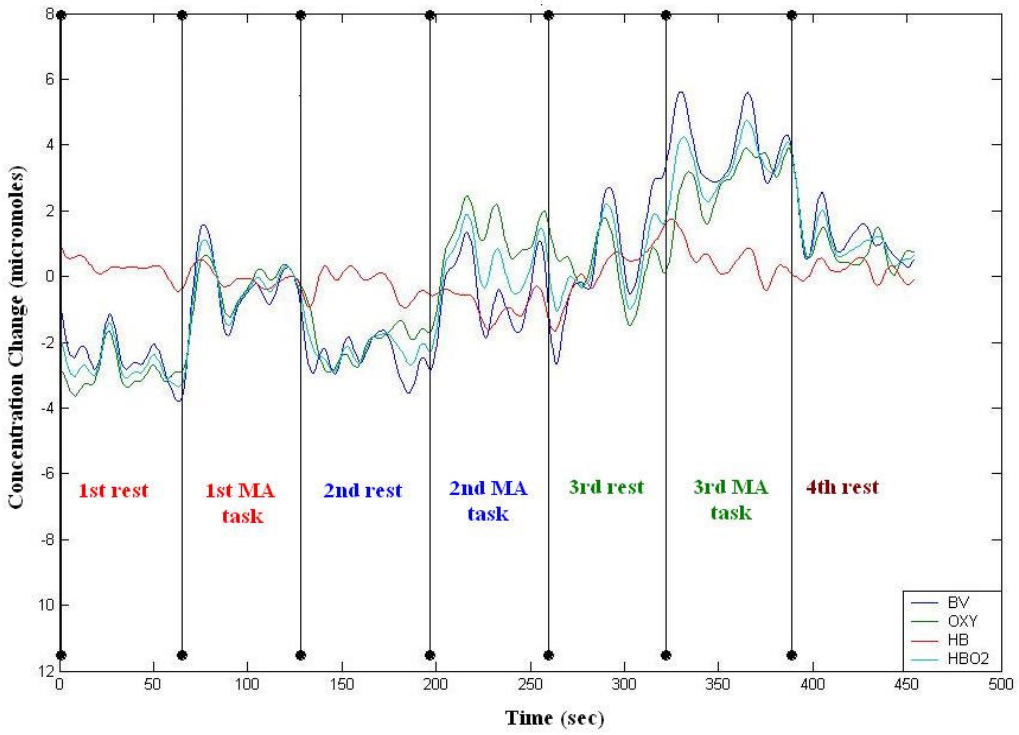

Figure 5.2 An example of fNIRS signals for an individual healthy subject's single detector during MA task.

In order to eliminate the noise in the signals, the data were first digitally low pass filtered at 0.08 Hz and then detrended. Mean values of the fNIRS derived signals were calculated for each level of complexity (mean of 60 seconds of fNIRS data for each level) and resulting mean values were analyzed by one-way ANOVA. Active detectors were identified from the ones whose mean values were significantly different ($p < 0.05$) in all 3 level of complexity.

A laterality index (LI) was calculated based on the oxy-Hb levels. Mean values of the active detectors from the left and right hemispheres were independently averaged for each level of complexity. Averaged value of the left detectors were subtracted from the averaged value of the right detectors and the result was normalized to the sum of the averaged values (LI = [right-left]/[right+left]). LI>0 shows higher activity of the right PFC, whereas LI<0 shows higher activity of the left PFC. LI values greater than 1 and less than -1 were ignored for each level of complexity . Finally, LI was plotted against percentage of wrong answers. In order to test the significance of LI and behavioral data, t-test was applied and these were compared with each other.

6. RESULTS AND DISCUSSION

6.1. Behavioral Data

The answers of all of 16 healthy subjects were recorded and evaluated as false or correct. Means of performances (after any performance error greater than 80% was eliminated) were 15 ± 17% for 1st level, 28 ± 14% for 2nd level and 40 ± 21% for 3rd level ($p=0.0012$). Means of performance for each complexity level show an increasing trend from 1st to 2nd ($p=0.00338$) level and 1st to 3rd level ($p<0.0001$) but not from 2nd to 3rd complexity level ($p=0.274$). This could be due to the fact that actual subtraction procedural complexity arises when a 2 digit number is being subtracted from a 3 digit number but when the same number is subtracted from a 4 digit number; the first digit of the 4 digit number is not used at all in the subtraction process but only memorized. Hence there is no significant difference in the performance between the 2nd and the 3rd complexity levels.

The answers of all of 16 migraine patients were also recorded and evaluated as false or correct. Means of performances (after any performance error greater than 80% was eliminated) were 20 ± 13% for 1st level, 42 ± 17% for 2nd level and 36 ± 13% for 3rd level ($p=0.000803$). Similar to the controls, means of performance for each complexity level show an increasing trend from 1st to 2nd ($p=0.000582$) level, from1st to 3rd complexity level ($p=0.005782$) but not from 2nd to 3rd complexity level ($p=0.274$). Figure 6.1 shows the performances of both groups.

There was no significant difference between the two groups performance results for 1st and 3rd sets. Only significant difference was observed in 2nd set between these groups ($p=0.0205$).

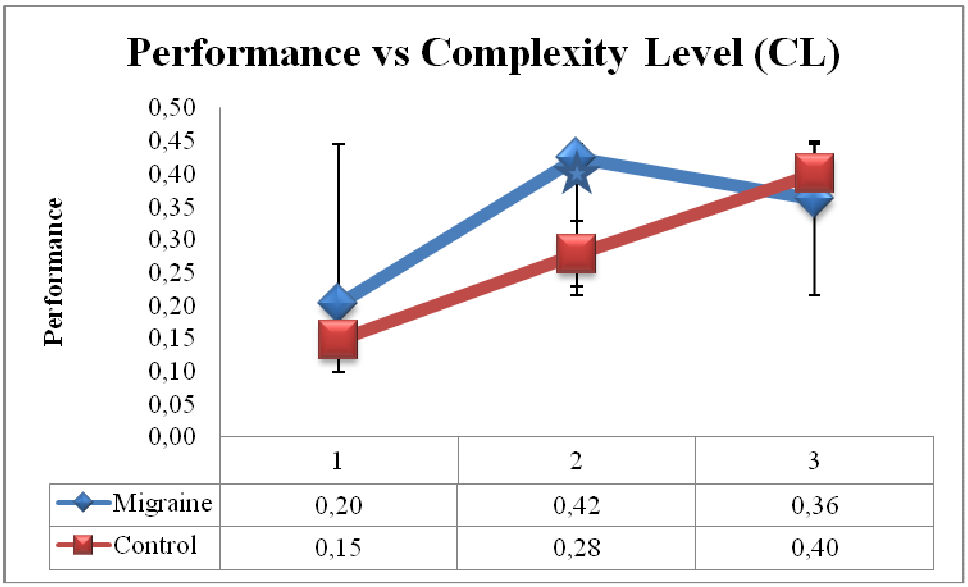

Figure 6.1 Performance of the migraine and control groups are plotted with respect to complexity level. Star is showing the significance between two groups. Standart deviations for each task are also shown with STD bars.

Means of number of questions attempted (NQA) were 12.1 ± 4. for 1st level, 5.7 ± 2.1 for 2nd level and 4.9 ± 1.4 for 3rd level (p=0). We also calculated the mean response time (MRT) in seconds for each level of complexity by dividing the number of questions answered during the 60 seconds. MRT for 1st level of complexity is 6.08 ± 4.09 sec, 12.47 ± 6.35 sec for 2nd level of complexity, and 13.77 ± 5.75 sec for the 3rd level of complexity (p=0.000565). Paired comparison of the MRT shows a significant difference between the 1st and 2nd level (p=0.000185) and 1st and 3rd levels (p<0.0001) but not between the 2nd and 3rd levels (p=0.08).

Means of number of questions attempted (NQA) for migraine patients were 10.9 ± 3 for 1st level, 5.9± 2 for 2nd level and 4.3 ± 1.2 for 3rd level (p=0). MRT for 1st level of complexity is 6.26 ± 2.16 sec, 12.29 ± 5.53 sec for 2nd level of complexity, and 15.94 ± 5.36 sec for the 3rd level of complexity (p=0.000187). Paired comparison of the MRT shows a significant difference between the 1st and 2nd level (p=0.000318) and 1st and 3rd levels (p<0.0001) but not between the 2nd and 3rd levels (p=0.07). Figure 6.2 shows decrease of NQA for both groups and Figure 6.3 displays increase in MRT for both groups with respect to complexity level. Overlapping increases in MRT and decreases in NQA show the similarity of the two groups in their cognitive abilities.

There was no significant difference between the two groups for both NQA and MRT.

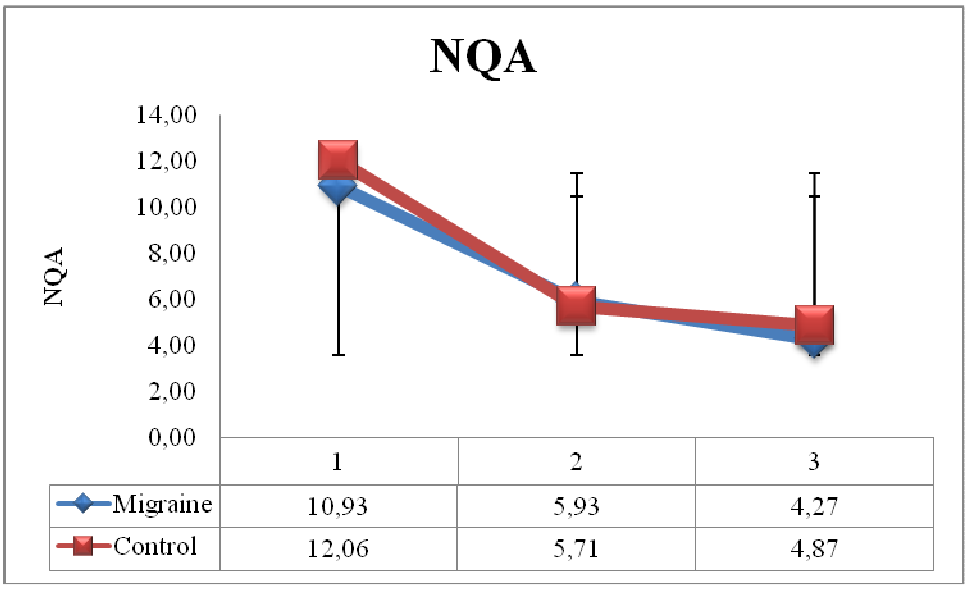

Figure 6.2 Number of questions answered for both groups is shown. Standart deviations for each task are also shown with STD bars.

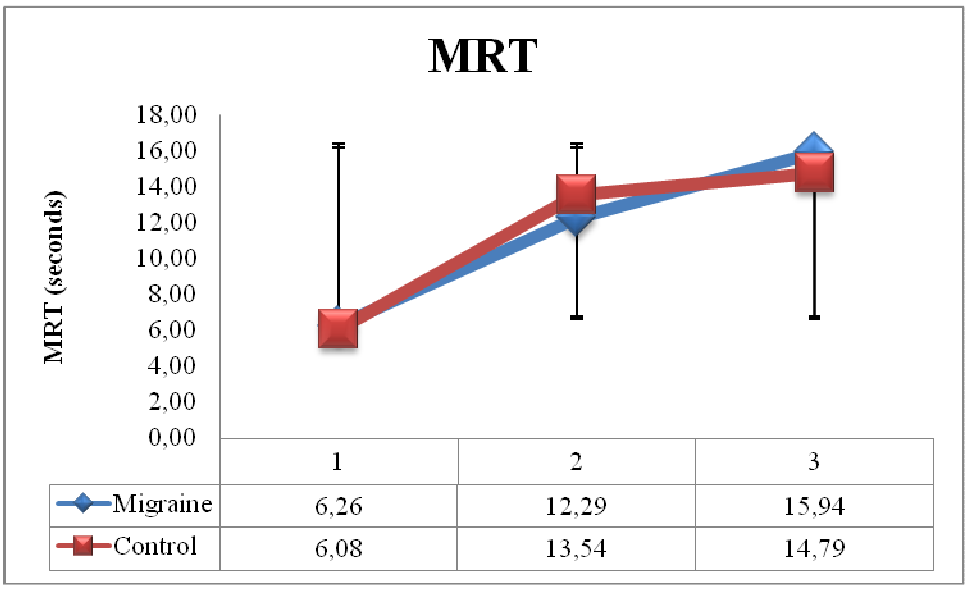

Figure 6.3 Mean reaction times with respect to test complexity are shown for both groups. Standart deviations for each task are also shown with STD bars.

We also computed the Work Load according to the equation below:

$$WL = \frac{MRT}{NQA}$$

Mean WL values of control group for the 1st level was calculated to be 0.88 ± 1.59, 3.22 ± 3.77 for the 2nd level, and 3.67 ± 3.57 [sec/questions] for the 3rd level (p=0.0349). Similarly the WL also showed significance between the 1st and 2nd levels (p=0.013), 1st and 3rd levels (p=0.00016) but not the 2nd and 3rd levels (p=0.16) as expected.

Mean WL values of migraine patients for the 1st level were calculated to be 0.61 ± 0.32, 2.18 ± 1.10 for the 2nd level, and 4.06 ± 2.17 [sec/questions] for the 3rd level (p<0.001). Similarly the WL also showed significance between the 1st and 2nd levels (p<0.0001), 2nd and 3rd levels (p=0.009) and 1st and 3rd levels (p=0.00026).

Figure 6.4 shows the increase in workload for each observed group. They do not show statistical significance, but they act in similar trends.

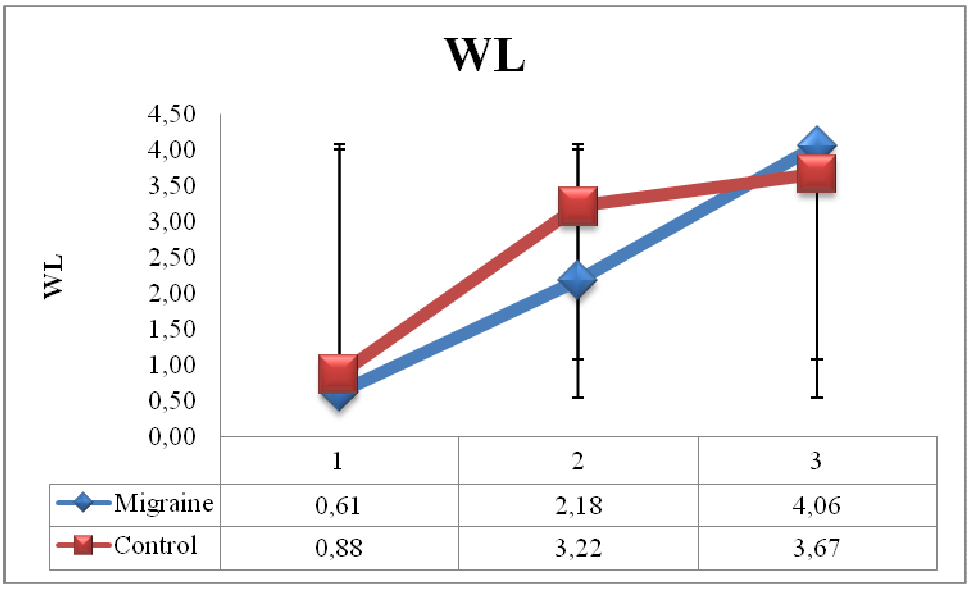

Figure 6.4 Workload of both groups with respect to complexity level is shown. Standart deviations for each task are also shown with STD bars.

These analyses confirmed that our manipulation of complexity was effective. It should be noted that performance can be modulated by imposing a time limit for each level of complexity (60 seconds) which is known to generate a stressful condition. Nevertheless, it has been shown previously that even though MA task induces stress, it does not debilitate performance drastically.

Working memory uses central executive and attentional resources to solve arithmetic problems [53]. However, for single digit or more difficult problems, when subjects retrieve the answers from their memory, central executive is no more essential. When subjects use counting for solving single digit problem for derivation of transient results, involvement of the phonological loop is seen. The third component of working memory is visuo-spatial sketchpad (VSSP), which is thought to be involved in visualizing the problems on a mental blackboard and this could lead to future work for researchers. Although Destefano et al designed a much more complex mental arithmetic procedure including addition of single digit or multi-digit with the value of the carry from one column to the next is one, we used the subtraction with similar properties [53]. In order to eliminate the possibility of solving the problems with only memory retrieval process, we designed our mental arithmetic task including 3 complexity steps, starting from the subtraction of 2 digit number from 2 digit number as the 1st step, then subtraction of 2 digit number from 3 digit number as the 2nd step, and subtraction of 2 digit number from 4 digit number as 3rd and the last step. Considering the 3 components of the working memory, as problems involve increasing number of digits in questions more of the central executive resources might be involved as well as the retrieval process [54]. Our MA task design was based on increasing the memory load by increasing the complexity of the steps. As the most difficult step was the 3rd step, it could be said that the executive functions are mostly used in this step, then in 2nd and 1st steps respectively. Concerning the remaining 2 other components of the working memory, the increase in the complexity level of the task is followed by the increase in usage of the phonological loop to maintain interim results and the usage of visuo-spatial sketchpad to visualize the information on mental blackboard.

In this study, first of all, performances of mental arithmetic task, which is calculated as the wrong answer percentages, increase in parallel to the increase in the complexity of the questions. This explains the rise in the memory load as a reaction to the increase in the complexity of the task. It should be considered that working memory load

and its increase should act within the brain's ability and limitations of calculation. Similar to the findings of Mattay et al, our performance results represent that subjects give more wrong answers as task complexity gets higher [55]. Second common finding with Mattay et al is that response times (MRT in this case) show a rising trend as subjects try to solve the more difficult problems [55]. According to the results, it is seen that mean reaction times do not show a big change from 2^{nd} to 3^{rd} step, when compared to the change from 1^{st} to 2^{nd} step. As well as this, performance results also show the same difference between complexity steps. These findings may be pointing to concentration loss in subjects.

6.2. Brain Activation and Imaging Data

The oxy-Hb-fNIRS data show a linear increase for each significant detector when plotted against performance. As performance increases, level of oxy-Hb also increases in most of the detectors. Figure 6.5 visualizes the relationship between PFC activation and mental arithmetic performance of both control group and migraine patients.

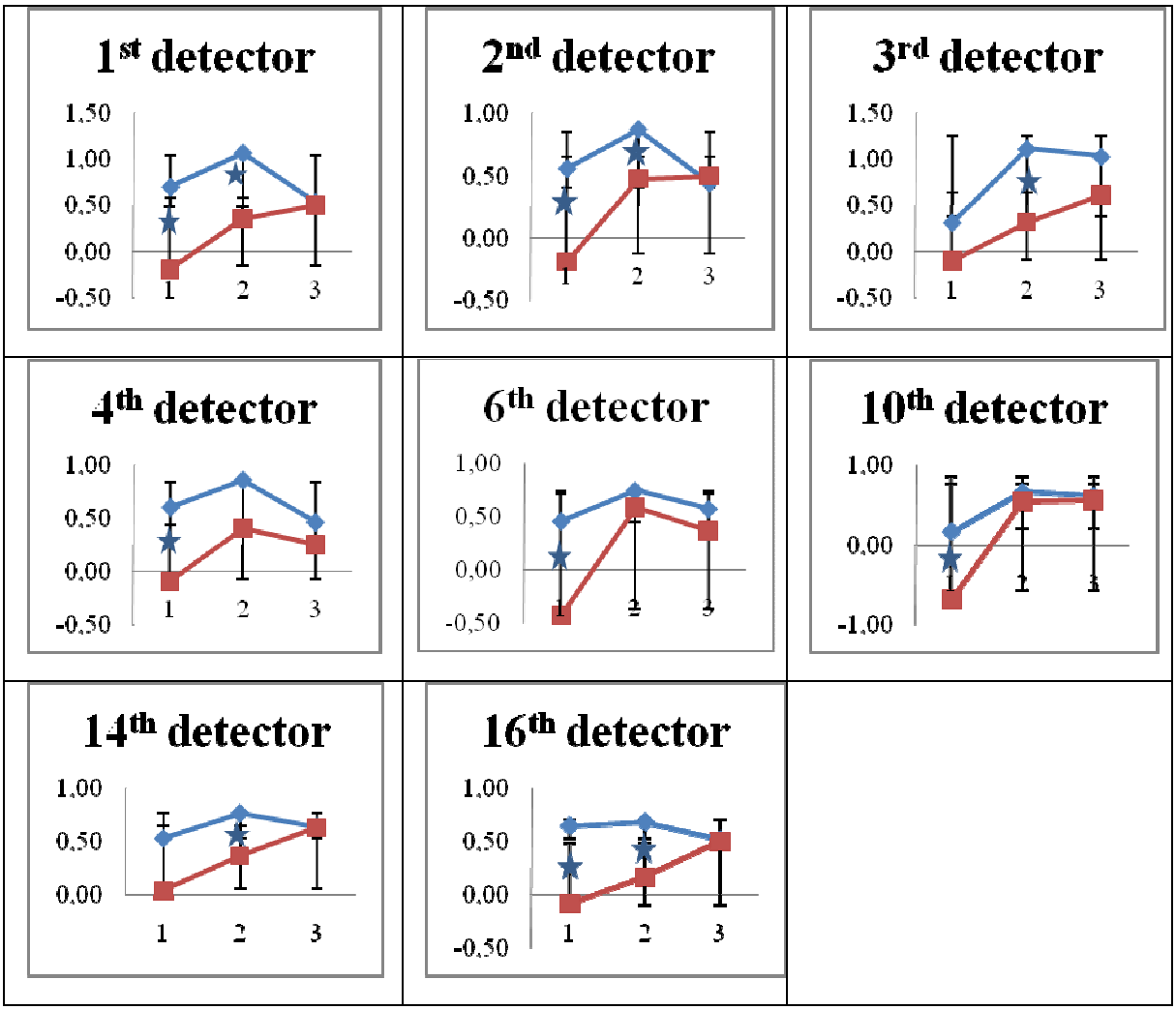

Figure 6.5 Red is representing the control group, blue represents the migraine patients. Mean change in oxy-Hb [microM] vs. level of complexity plots for all the statistically significant detectors. Detectors 1-8 lie on the left forehead while 9-16 are on the right of the forehead. As reported in previous studies, detectors 14 and 16 that correspond to right dorsolateral prefrontal cortex show a linear increase on oxy-Hb with respect to complexity of the task in control group. Stars are showing the significance between the complication steps of two groups. Standart deviations for each task are also shown with STD bars.

Rise of oxy-Hb level in several of the active detectors in Figure 6.5 can be related to complexity of MA levels by forcing subjects to pay more attention on questions, therefore forcing their PFC to work harder. This forcing effect increases the blood volume of the PFC vessels and oxy-Hb. Although oxy-Hb concentration levels can differ greatly from one individual to another, fNIRS data could still be a marker for screening the level of attention paid during a cognitive task.

The negative results seen in the oxy-Hb of the controls during the 1st level represents the decrease in the amount of oxy-Hb from baseline. This may be due to the fact that during this 1st level, the oxygen consumption exceeds the immediate delivery of blood flow to these regions leading to a negative oxy-Hb concentration change. Subtraction of a 2 digit from a 2 digit number may not be as stressful as compared to other complexity levels. Hence, rise in heart rate and blood pressure may not be as high as the others and the demand of neurons working quite fast and efficiently may not have involved large cerebral blood flow (CBF) recruitment. After the first rest period, the brain slowly picks up on the blood flow and is ready for the second level. It is in this level that we see positive oxy-Hb change. We also observe a positive difference between the end and the beginning of the test in the concentration of oxy-Hb, meaning that during the MA task, the hemodynamic response clearly builds up for healthy patients, the control group.

Analyses of fNIRS data of both groups demonstrate increases in the concentration of oxyhemoglobin in line with the increase in the complexity of the task. This result is concordant with the findings of the study done by Izzetoglu et al, which applied an n-back working memory task that has three complexity steps as we had in our study [56]. They also showed the rising oxy-Hb concentration according to the procedural complexity. However, both in their and our findings, a small decrease were also observed when passing from 2nd level to 3rd level. Similar to fMRI findings, the significant increase in [oxy-Hb] between 1st and 2nd levels shows a positive correlation between increasing workload and the oxygenation in dorsolateral prefrontal cortex [57]. Assuming that all subjects attended each step with the nearly unchanged concentration, the increase in the wrong answer percentage and the memory load increase (i.e. complexity of the task) can be correlated. It can be said that the insignificant [oxy-Hb] change and performance decrease during 2nd and 3rd levels could mean that at the end of 2nd task vessels reach their upper limits of diameter and cannot dilate any more as they did in 1st level. Moreover, this peak point is followed by an attention loss leading to worsening of the performance.

Concerning the results shown in Figure 6.5, there is an obvious difference in the initial oxy-Hb concentrations of migraineurs and healthy subjects. We will try to address the peculiar finding with respect to the neurovascular coupling disorder theory of migraine pathophysiology. Mental arithmetic test is used as a stress inducer in many brain studies and also in fNIRS studies done by Tanida et al [27, 28]. Stress, caused by the mental

arithmetic test is regulated by the autonomic nervous system (ANS). Analysis and studies on ANS showed that prefrontal cortex, particularly the right PFC, is activated during negative emotional states, dominating sympathetic activation of ANS during stress-induced mental tasks. It is also mentioned in the study of Tanida et al that in several previous EEG findings increases in heart rate and blood pleasure were also shown to be related to the sympathetic activity of right PFC [28]. Hence, we can deduce that as an autoregulation system, the sympathetic nervous system controls the vessel diameter. Simply formulated ANS also has regulatory control over the vessel dilation-constriction process. When a mental arithmetic test is introduced to a subject, the stress inducer task stimulates the ANS. As a result of the stimulation, sympathetic response is generated as an increase in heart rate, blood pressure, therefore also in blood flow and in vessel diameter as known as dilation. We believe that as stress factor is increasing step by step and getting effective in each complexity level, the hemodynamic changes also increase in parallel. Since the neuron-vessel interaction is explained by the neurovascular coupling, and we claim that migraine is a neurovascular coupling disorder; migraineurs' brain cannot keep in step with sequential vasodilation. Once a migraineur's brain is exposed to stress or cognitive activity, the coupling dysfunctioning shows itself as a sudden and big response resulting in a sudden vasodilation and blood flow. Therefore, this hypothesis explains the difference in the initial concentrations of oxy-Hb.

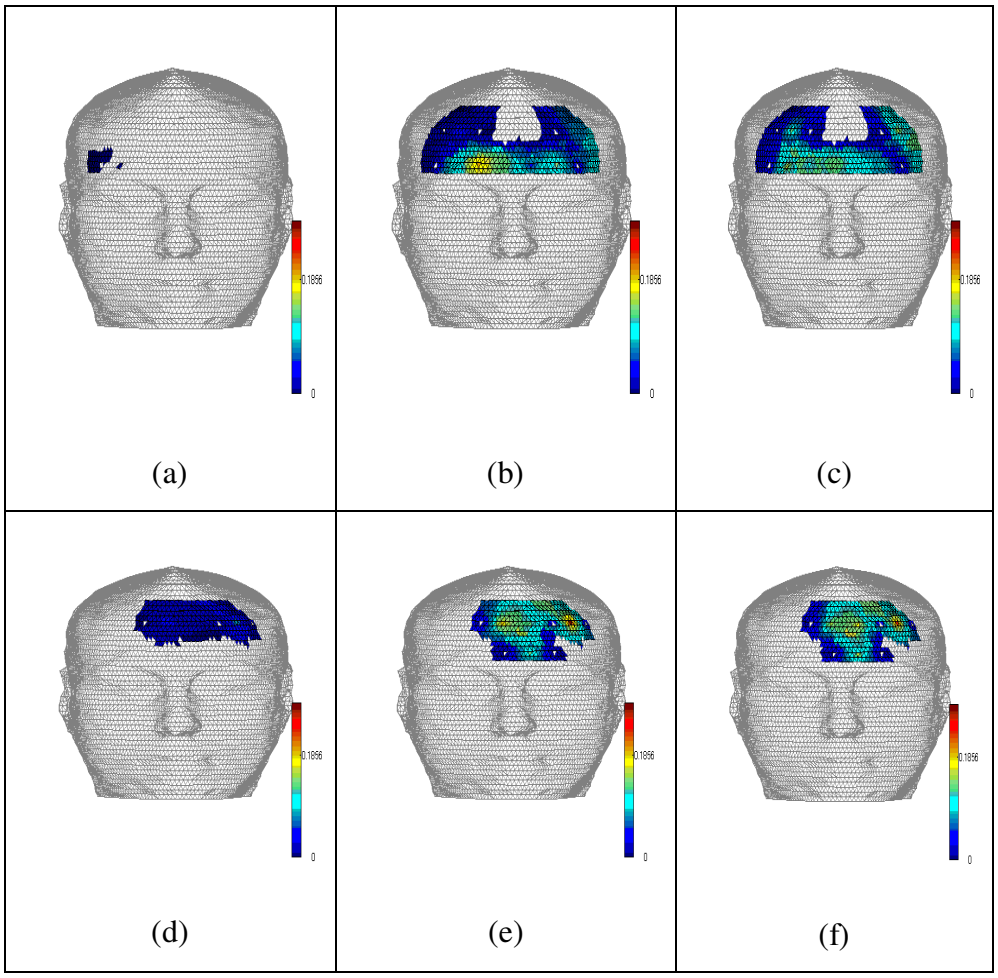

Figure 6.6 a-c HbO_2 topography of controls, d-e HbO_2 topography of migraineurs for increasing complexity level (level 1-3) Negative values are ignored.

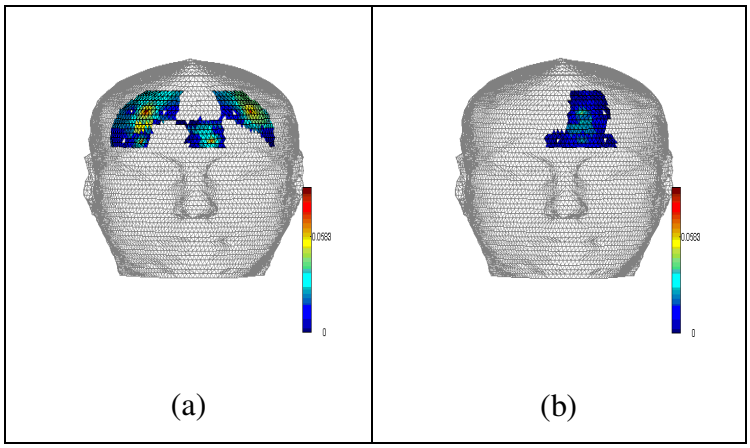

Figure 6.7 Interference of level 3 from level 2 (difference of c-a in Figure 1) for controls (a) and for migraineurs in (b). Negative values are ignored.

Another interesting issue, as seen in Figure 6.5 and figure 6.6, is the difference in the concentration changes from 1st to 3rd in control group compared to the migraine group. Although the initial [oxy-Hb] values are different for both groups, resulting values of [oxy-Hb] are nearly the same. We believe that the vessels have reached to their maximal dilation diameter. As vessels' volume change is limited to their elasticity (compliance) and initial diameter, once they are dilated enough to reach their maximum volume, they cannot continue this process any longer and either they keep their position for a while or recover back to their original size. Therefore, inferring from our results, we can say that a big part of the vaso-response takes place between the first two levels, but at the end of the 2nd task, either the vessels reach their climax resulting in a decrease in [oxy-Hb] or their dilation amount is not as much as to the first level. Amount of dilation directly affects the blood flow and the concentration of the incoming blood. Therefore differences or stabilities in dilation can be thought in the same manner with blood flow.

6.3. Work Load and Asymmetry of Brain Activation

Data show an asymmetric activation between the left and right sides of the PFC related to the performance of subjects. This asymmetry can be quantified with the laterality index. It is seen that activation shifts to the right side as task becomes harder and as performance increases for both healthy subjects and migraine patients.

As seen in Figure 6.8, right dominant prefrontal activity moves to the left PFC in 2nd task and becomes dominant in right PFC in 3rd task as work load increases in healthy subjects. This result is consistent with the results of previous studies [27, 28]. However, left dominant PFC activity decreases as task complexity increases in migraineurs and but stays in the left PFC till the end of the task.

There was no significant difference between the two groups' LI results.

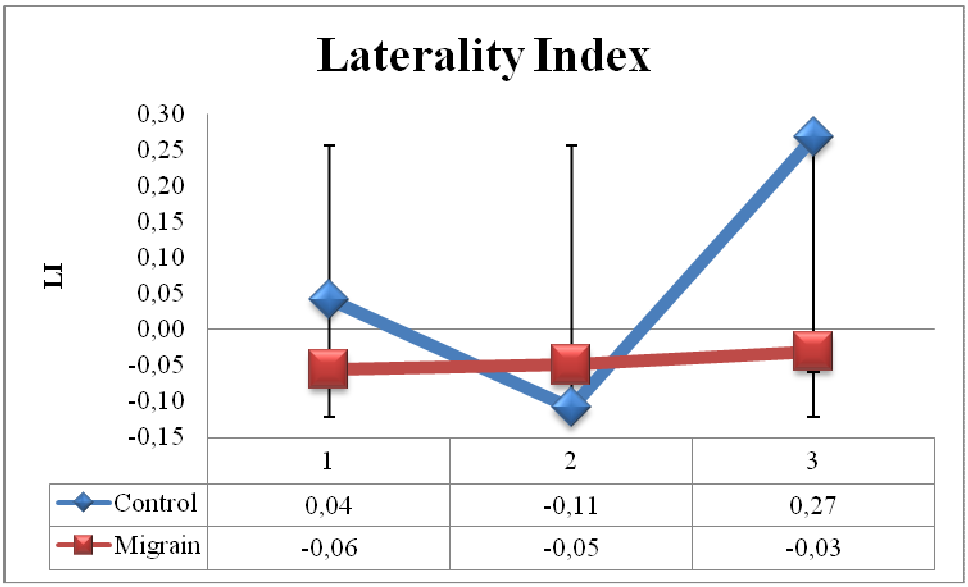

Figure 6.8 Changes in laterality index (LI) for control group and migraineurs are plotted. Standart deviations for each task are also shown with STD bars.

LI values between -1 and 1 are eliminated after [27, 28]. Work load data were also truncated by eliminating the smallest and largest numbers while the corresponding LI values were also discarded. Figure 6.9 and Figure 6.10 represent the LI and WL results of healthy subjects and migraine patients respectively.

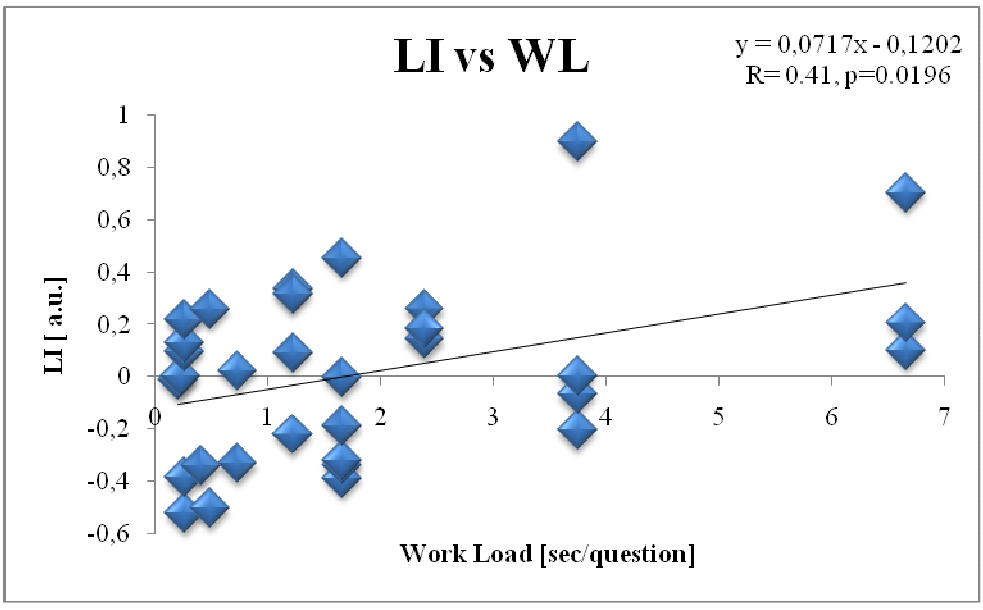

Figure 6.9 Laterality index and work load during activity (for control group) is plotted.

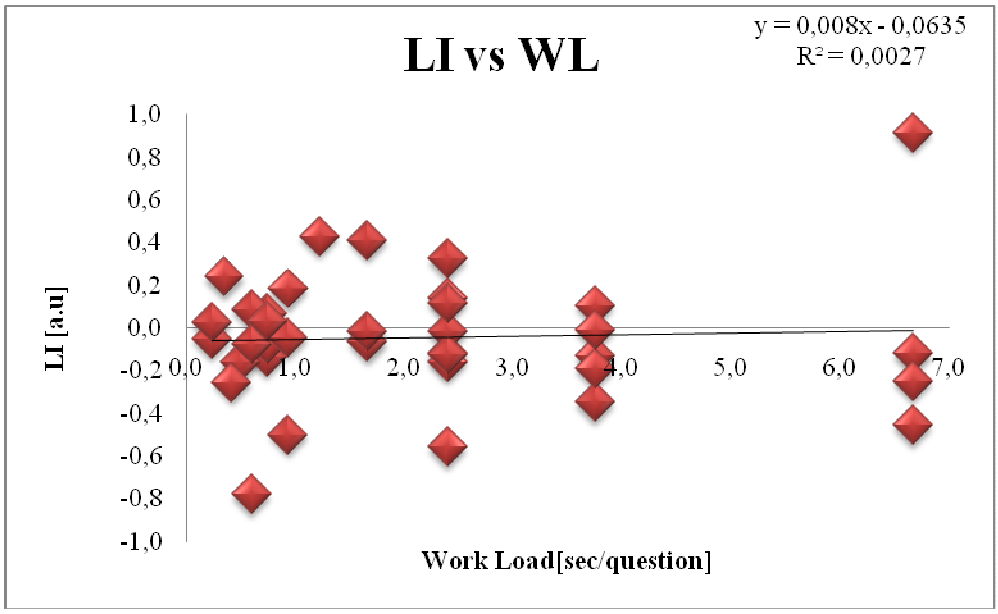

Figure 6.10 Laterality index and work load during activity (for migraine patients) is plotted.

It can be said that the behavioral data (WL in this case) and asymmetry of the hemodynamic response can be related, meaning that performance of the subjects affect the activation amount of the PFC.

Evaluation of the lateralization indices shows consistent results with the similar two studies done by Tanida et al, that the mental arithmetic task was found to be right lateralized [27, 28] In this study they also used a pulse-oximeter to measure the heart rate (HR) of the subjects. They found right lateralization for the high HR and left lateralization for the low HR group. MA task is known to induce a negative emotional effect (i.e. stress inducer) which is known to be localized to the right side of the prefrontal cortex. In a recent study we showed that during simultaneous measurement of heart rate and fNIRS data for MA task, there is always a right lateralized activity. However, for high HR increase group, there appears a larger area of activation on the right dorsolateral prefrontal cortex [58].

Laterality of the brain activation increases as test gets harder meaning that work load is increasing. The number of the digits and the complexity of the test are organized to get bigger in order to force the PFC to react efficiently. Therefore, the results show that as work load gets harder, brain activation is more right-lateralized.

The research about the induced prefrontal activity by mental arithmetic task of human brain of migraine patients and healthy subjects would lead to the understanding of the possible disorder and pathophysiology underlying migraine. There should be other neuroimaging studies including PFC and other brain regions to lighten the differences between healthy and diseased (migraine) brain functions to come to an explanatory and correct conclusion of migraine.

6.4 Final Word

The control of the autoregulation of the cerebral blood flow can be performed by three major mechanisms: myogenic mechanisms, local metabolic factors and innervations of the cerebrovascularature by the branches of the autonomic nervous system (ANS). It has been observed that this autoregulation is modulated rather than being controlled by the sympathetic innervations. Due to this, an increase in blood pressure causes increase in perfusion according to the rise in the sympathetic activity and oppositely a fall in the sympathetic activity will cause a drop in perfusion. It has been shown that, under several physiological and neuropsychological tasks, migraineurs have an impairement in neurovascular reactivity due to a sympathetic hyper-responsiveness [59, 60, 61]. They show exaggerated and unhabituated hemodynamic response to these tasks compared to controls.

7. CONCLUSION

The difference between the migraine patients and healthy subject in brain hemodynamics examined with mental arithmetic task containing 3 complexity steps was evaluated with the participation of 16 healthy subjects and 16 migraine patients. Data obtained with fNIRS from 16 detectors of both group of subjects were statistically analyzed and compared. The performances, response times, work load and laterality indexes for each groups were evaluated. The LI of each groups were calculated considering the Hb oxygenation at left and right prefrontal cortex of both groups with a formula of LI=(Right-Left)/(Right+Left). Increase in performances, response times, work load and oxy-Hb were consistent with the increases in procedural complexity (i.e. work load). The mean performances and response times were similar for each group. The activation in the PFC measured with the [oxy-Hb] changes showed statistically significant differences between migraineurs and control group, as unregulated activation in migraine patients and steadily increasing activation in healthy subjects. As a result of the LI analysis, mental arithmetic task showed a right dominant PFC activity for the control group, whereas a left dominant PFC activity, close to symmetry, for the migraine group. The results of this study could be a neuroimaging study demonstrating the fNIRS findings showing that migraine is a neurovascular coupling dysfunction.

Neurophysiological and neuroimaging studies have discovered that between migraine attacks, there are some abnormalities of cortical information processing. Cappola et.al. claim that the reason for the next attack is the increas in responsivity to external stimuli in migraine, i.e. a deficit of habituation during stimulus repetition, of which the metabolic correlate was recently demonstrated in functional neuroimaging studies. The reason underlying this phenomenon is still under debate. In theory, there may be two possible reasons: increased cortical excitability (and homosynaptic facilitation) or decreased intracortical inhibition (heterosynaptic inhibition) [62].

Future studies on understanding the pathophysiology of migraine can be performed by using an ANS blocker drug to prove the possible role of ANS. As well as this, pulse-oximeter should be used during experiments in order to record the heart rate of the subjects.

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