FMUC FACULDADE DE MEDICINA UNIVERSIDADE DE COIMBRA

THE ROLE OF EEG AS A BIOMARKER TOOL IN ASSESSING PLASTIC CHANGES INDUCED BY TRANSCRANIAL MAGNETIC STIMULATION IN STROKE PATIENTS

Thesis presented to Faculdade de Medicina da Universidade de Coimbra to fulfill the requirements of Master of Science degree in Biomedical Research

by

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Never go backward. Attempt, and do it with all your might. Determination is power.

Charles Simmons

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Esta Tese de Mestrado representa um marco importante na minha vida e quero agradecer a todos aqueles que deram a sua contribuição, pois sem eles, este projecto não teria sido possível.

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Abstract

Stroke is the leading cause of long-term disability. It occurs when the blood supply to the brain is disrupted by cerebrovascular disease, which can lead to permanent damage, depending on the duration and extent. After stroke, neuroplasticity occurs and this is one of the main factors that one could potentially use to overcome the caused damage. One of the techniques which has been able to modulate the brain's plasticity and has been achieving promising results is transcranial magnetic stimulation (TMS).

In this study we used the continuous theta burst stimulation (cTBS), a protocol that inhibits the hemisphere in which it is applied, so that the other hemisphere becomes more excited. We had two main objectives in this study, first to characterize physiological patterns in healthy subjects and then to study their potential relevance in the context of stroke. For one session, cTBS was delivered over the unaffected hemisphere of the patient. Healthy subjects were divided in two groups: one group received the cTBS protocol on the left hemisphere and the other group received it on the contralateral hemisphere. Thus, the aim of this study is to understand the brain's physiology before and after cTBS, to provide a possible rehabilitation approach to stroke patients with motor deficits; the other aim is to know if the cTBS protocol when applied on the dominant or the non-dominant hemisphere has the same results.

To understand the brain's changes before and after the TMS we used the electroencephalogram (EEG). EEG at high recording density was used to evaluate the brain's activity at rest and to analyze the event-related desynchronization (ERD) and synchronization (ERS) of electrophysiological motor biomarkers (e.g. mu rhythm, beta activity) when the subjects performed two different types of movements, one with arms and the other with hands.

Our results showed that cTBS affected the brain's physiology and biomarkers of motor activity. When applied to the dominant or non-dominant hemisphere cTBS protocol has showed different aftereffects. For the stroke patients the results were matched to one control that received cTBS on the same hemisphere. The patient and the matched-control showed similar results for complex movements (hand tasks); while, for simpler movements (arm tasks) they behaved differently, except for the right arm. We hypothesized that this difference on the arm tasks results could have occurred because the patient activated brain areas that are normally recruited in more demanding tasks. Despite the results observed it will be needed more patients and additional studies to have more reliable conclusions.

Keywords: Stroke; Electroencephalogram (EEG); Continuous theta burst stimulation (cTBS); Event-related desynchronization (ERD); Event-related synchronization (ERS); Alpha rhythms; Beta rhythms

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Symbols and Abbreviations

AC- Anterior Commissure

ADM- Abductor Digiti Minimi

µV- Amplitude

APB- Abductor Pollicis Brevis

CHUC- Coimbra Hospital and University Center

CNS- Central Nervous System

CT- Computed Tomography

cTBS- Continuous Theta Burst Stimulation

EEG- Electroencephalogram

EMG- Electromyography

ERD- Event-Related Desynchronization

ERS- Event-Related Synchronization

Hz- Frequency

FAS- Functional Ability Scale

FDI- First Dorsal Interosseous

fMRI- Functional Magnetic Resonance Imaging

GABA- γ-aminobutyric acid

HF-rTMS- High Frequency Repetitive Transcranial Magnetic Stimulation

ICF- Intracortical Facilitation

ICNAS- Institute of Nuclear Sciences Applied to Health

iTBS- Intermittent Theta Burst Stimulation

LF-rTMS- Low Frequency Repetitive Transcranial Magnetic Stimulation

LICI- Long-Interval Intracortical Inhibition

LTD- Long Term Depression

LTP- Long Term Potentiation

M1- Primary Motor Area

MI- Motor imagery

ME- Motor execution

MT- Motor Threshold

MEP- Motor Evoked Potential

MRS- Modified Rankin Scale

MRI- Magnetic Resonance Imaging

NIHSS- National Institutes of Health Stroke Scale

PC- Posterior Commissure

PET- Positron Emission Tomography

pp-TMS- Paired Pulse Transcranial Magnetic Stimulation

rMT- Rest Motor Threshold

rTMS- Repetitive Transcranial Magnetic Stimulation

SMA- Supplementary Motor Area

SICI- Short-Interval Intracortical Inhibition

TBS- Theta Burst Stimulation

TCR- Thalamocortical Relay

TMS- Transcranial Magnetic Stimulation

TRN- Thalamic Reticular Nucleus

WMFT- Wolf Motor Function Test

1. INTRODUCTION

I am really thankful to be part of this research project. My background on neurophysiology gave me excellent opportunities to be involved in research throughout my academic years and such experience has allowed me to become even more absorbed in the neuroscience world. For me it is an honor to become part of a scientific community; to pursuit a career in research and experience the excitement and satisfaction of being in the neuroscience field. Stroke has a massive impact on the quality of life of individuals and is one of the most prevalent diseases in our society. Therefore, it is very motivating to have the opportunity to give my contribution in increasing the knowledge on this field.

This thesis was proposed in the discipline “Master's dissertation in neurobiology” as part of the second year of the Master Plan in Biomedical Research, Faculty of Medicine of the University of Coimbra.

The research project was carried out at the Institute of Nuclear Sciences Applied to Health (ICNAS), guided by the group of Professor Miguel Castelo-Branco and with the collaboration of the Stroke Unit of the Coimbra Hospital and University Center (CHUC).

Stroke is one of the most frequent causes of death and is a leading cause of disability. There are several strategies to deal with its consequences. However, there is a need of more effective approaches that can improve post-stroke quality of life. In this way, stroke rehabilitation emerged as a great theme for my research.

We applied transcranial magnetic stimulation (TMS), in a repetitive pattern, to healthy subjects and to stroke patients recruited from the CHUC hospital from five to nine days post-stroke, aiming at assessing the potential for motor function recovery of the upper-limb. We seek, if feasibility is proven, to sequentially randomize the stroke patients (1:1 ratio) into two groups: one group that receives TMS and the other receiving a placebo intervention (sham stimulation). We studied in healthy subjects and in stroke patients the changes in brain plasticity induced by this technique, with a paired-pulse paradigm. Also, to help understanding the mechanisms underlying the action of the continuous theta burst stimulation (cTBS) in potentially improving the upper-limb impairment, we evaluated motor biomarkers such as mu and beta rhythm, through electroencephalogram (EEG). For the healthy subjects we studied if the cTBS when is applied on the dominant or non-dominant hemisphere can have different results.

EEG of high density was placed on the head of the subjects and was monitored before and after transcranial stimulation. First, the brain activity at rest was recorded to evaluate the

physiological state. Then, to analyze the event-related desynchronization (ERD) and event-related synchronization (ERS) of electrophysiological biomarkers (e.g. mu rhythm, beta activity) the subjects performed two different types of movements (first, each upper-limb individually and then simultaneously): arm elevation (upward, hold and downward) and thumb finger opposition. The task consisted in six repetitions of 15 seconds for each move, with an interval between repetitions of 15 seconds. Between each block of movements was an interval of 1 minute.

This thesis is focused on the EEG preparation, performance and analysis on the functional reorganization of the motor system in stroke patients, before and after TMS. The main two goals is to understand the healthy subjects and the stroke patients respond physiologically to the inhibitory protocol; and an accessory goal is to find if the hemispheric dominance influences the effect of cTBS protocol.

2. THEORETICAL BACKGROUND

2.1 Stroke

Nowadays, stroke is one of the leading causes of adult disability in the developed countries. Stroke is a condition which affects the blood supply to the brain and it is a form of cardiovascular disease. It has been seen as an elderly disease, however it affects younger individuals as well. The incidence does increase with age, and approximately a quarter of all strokes happen in people under the age of 60. The neurological changes can be severe or mild, and depending on the extension and on the location of brain damage the person's recovery is uncertain (Lawrence & Brass, 1992) (Chino et al., 1994) (Jordan, 2004) (Amengual et al., 2014) (Park et al., 2014).

Nerve cells within the brain need an uninterrupted supply of blood, oxygen and glucose. The brain cells are also called neurons, and they are the basic functional unit of the central nervous system. If this supply is disturbed, the affected area can stop its function for a certain period of time. In an early period after stroke, injured neurons are vulnerable to permanent damage from hypotension, hyperglycemia, fever and other systemic perturbations. Depending on the severity of the impairment, the brain cells can die following a permanent damage because the neurons are not replaced. The movement and /or other functions will be affected because they are controlled by these brain cells. So, depending on which blood vessels and parts of the brain are affected, the symptoms from a stroke can vary (Lawrence & Brass, 1992) (Graham & Hickey, 2002) (Hossmann & Heiss, 2009) (Jordan, 2004) (Ángeles Fernández-Gil et al., 2010).

To understand the signs and the symptoms from a stroke and how they can be different from patient to patient it is necessary to understand the brain topology of the lesions.

The brain is composed of 100 billion neurons and each one may connect to thousands of other brain cells. The neuron is composed by a cell body from whose surface projects one or more processes called dendrites. These dendrites receive information (electrophysiological impulses) from other neuron and conduct the information toward the cell body. These neural impulses in form of action potentials travel long distances through a tube called axon. Normally, each neuron has only one axon and it may have branches called axon terminals. A scheme is represented on figure 1, where it is seen that the axon comes off the cell body at the axon hillock and conducts the action potential to

the axon terminal. The communication with other neurons is achieved by synapses, in the process of neurotransmission. These connections regulate and control body movements, mediate thought and language and interpret all sensations (Lawrence & Brass, 1992) (Jordan, 2004) (Ángeles Fernández-Gil et al., 2010).

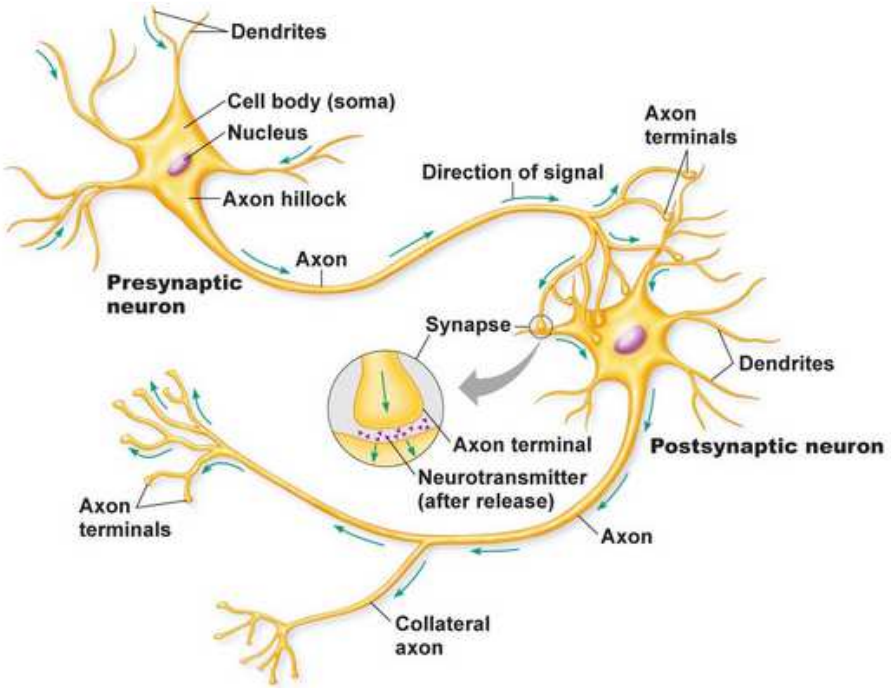


Figure 1. Typical neurons receive input signals (action potentials) in the dendrites or on the cell body and send signals down the axon toward other neuron (Stanfield & L, 2011).

The brain has a high metabolic rate, it uses about 25 percent of the body's oxygen and 70 percent of glucose. If the blood supply is interrupted for 30 seconds the person will be unconscious and if this interruption lasts more than four minutes, a permanent brain damage may follow (Lawrence & Brass, 1992) (Ángeles Fernández-Gil et al., 2010). Figure 2 shows the major arteries which have a main role to maintain a continuous blood flow and the brain's metabolic rate.

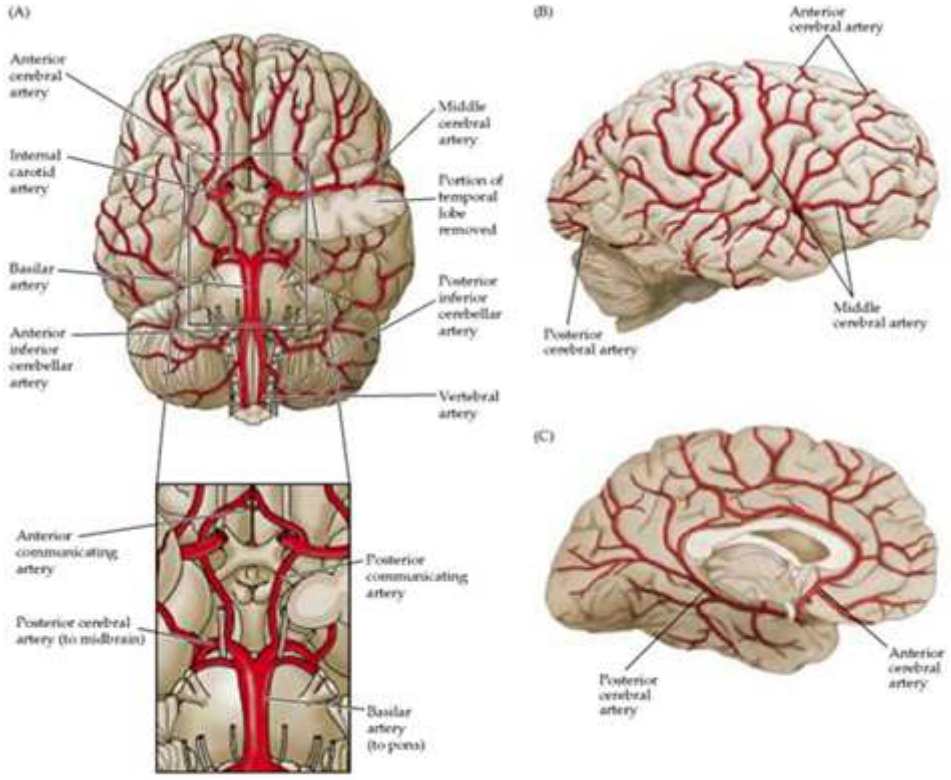


Figure 2. Major arteries supplying the brain. (A) Ventral view. The amplification shows the circle of Willis. (B) Lateral view. (C) Midsagittal view (Purves et al., 2001).

The brain can be separated into three parts: cerebrum, brainstem and cerebellum. The cerebellum is positioned at the back of the brain, underlying the occipital and temporal lobes of the cerebrum. This structure has an important role in motor control, and it may also be involved in cognitive functions, for example regulating fear, attention and language. Despite its important role in motor control, the cerebellum does not initiate the movement, but it contributes to precision, accurate timing and coordination. Another important function is to regulate neural signals such as input from sensory systems of the spinal cord and from other parts of the brain, and integrate these inputs through loops of interaction (Squire et al., 2002) (Nowinski, 2011).

In the cerebrum, the left and the right hemisphere are composed by outer gray matter which contains mainly nerve cell bodies, while inner white matter is made up predominantly of nerve fibers (axons). The right and left hemispheres communicate by a bundle of fibers called the corpus callosum. Each cerebral hemisphere has four different lobes: frontal, temporal, parietal and occipital (represented on figure 3). The frontal lobes control motor function, planning, personality emotions, speaking and writing (Broca's area). The temporal lobes are responsible for memory, hearing and understanding language (Wernicke's area). The parietal lobes are involved in interpreting language and

words, sense of touch, pain and temperature and spatial and visual perception. The occipital lobes process visual features, as color, light and movement. There are very complex relationships between these four different lobes of the right and left hemisphere. The right hemisphere is believed to be underlie creativity, spatial ability, artistic and musical skills. The left hemisphere controls speech, comprehension, arithmetic, writing and normally, in hand use and language it is the dominant hemisphere, for around 90 percent of people (Lawrence & Brass, 1992) (Jordan, 2004) (Ángeles Fernández-Gil et al., 2010) (Nowinski, 2011).

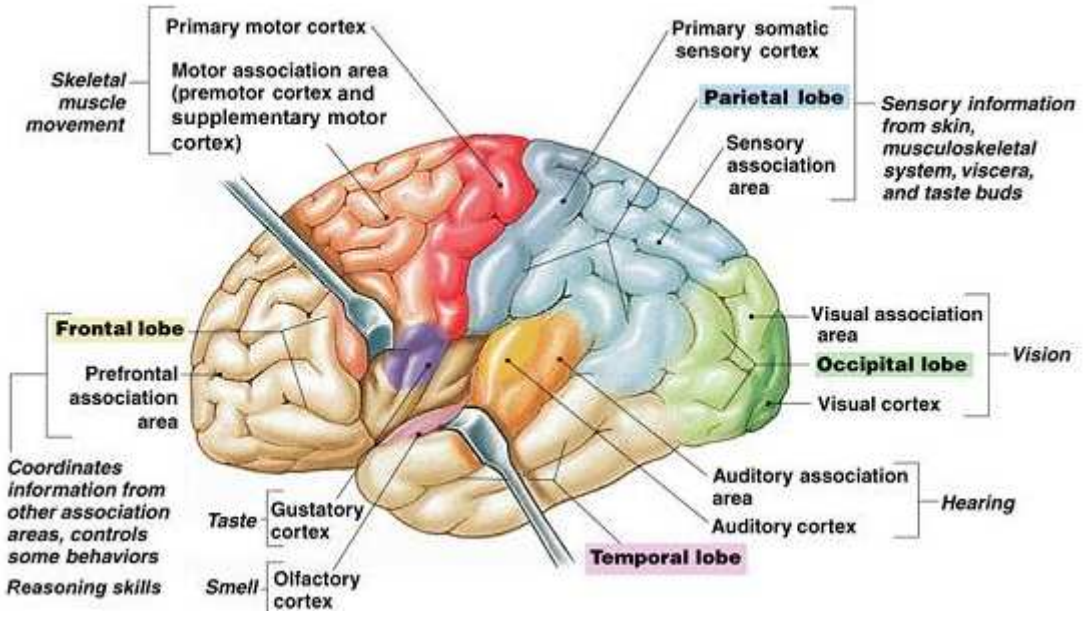


Figure 3. Different brain structures and functions (Martini, 2007).

The brainstem is in the posterior part of the brain and it serves a continuous connection with the spinal cord. It is composed by four parts: medulla oblongata (myelencephalon), pons (part of metencephalon), and midbrain (mesencephalon) and diencephalon. The main functions for which the brainstem is responsible are basic vital functions, for example heartbeat blood pressure, breathing, control of consciousness and sleep (Ángeles Fernández-Gil et al., 2010) (Nowinski, 2011).

Knowing that stroke is a condition which affects primarily the motor function it is important to describe how this can affect the normal brain function. The motor system is part of the central nervous system that is involved with movement and it consists in the pyramidal and extrapyramidal system. The pyramidal system or the corticospinal tract, ascends from the precentral gyrus of the cerebral cortex and it has the upper motor neurons. The upper motor neurons have a somatotropic arrangement which let us represent different parts of the body in certain areas of the cortex, the homunculus (“little

person"). The homunculus has specific parts of the cortex control specific for motor and sensory functions on the contralateral side of the body, which is exemplified on figure 4 (Ángeles Fernández-Gil et al., 2010).

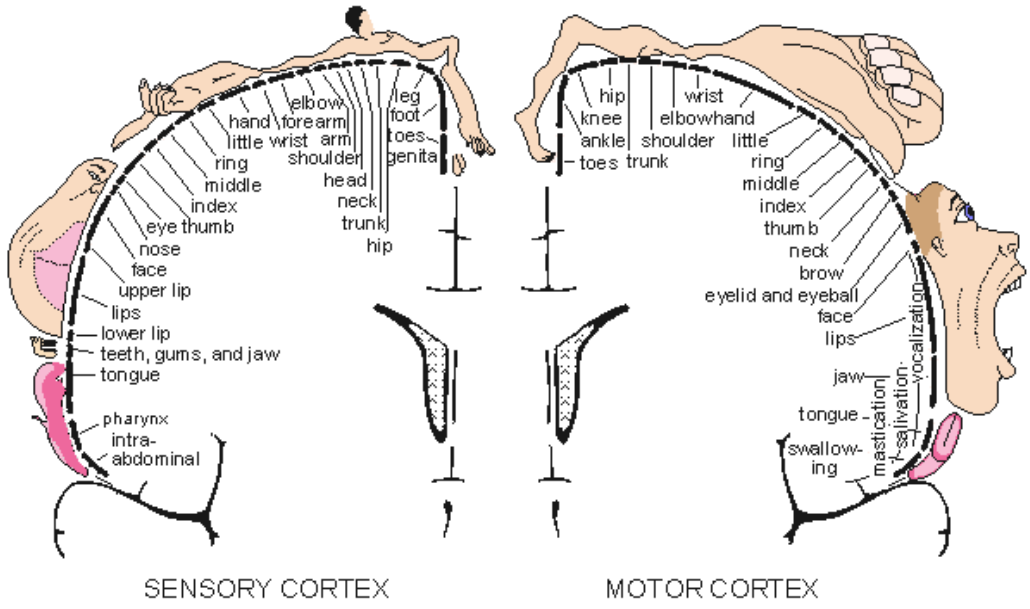


Figure 4. Homunculus: neural network's topographic specializations for somatosensory and motor cortices (Penfield & Rasmussen, 1950).

Some of the axons of the corticomesenphalic, corticopontine and corticobulbar tracts, cross the midline of the brainstem at the decussation of the pyramids to terminate at the motor cranial nuclei of the contralateral side. So, the cerebrum is divided into left and right hemisphere and normally, the left side of the brain receives sensations from the right side of the body and controls the movements also from the right side. That's the reason why, when a patient has a stroke in the cerebral cortex and some of these tracts are affected, it will result in a contralateral loss of motor function (Lawrence & Brass, 1992) (Ángeles Fernández-Gil et al., 2010).

2.2 Physiopathology of stroke

There are two types of stroke: ischemic and hemorrhage. The ischemic strokes are the most common and account for about 70 percent of all strokes. One common type of strokes is caused by a clot that blocks blood flow in an artery and is called cerebral atherothrombosis. The term cerebral infarction is used when the lack of oxygen results in death of brain tissue and permanent damage. Another type of ischemic stroke is the embolic, where a lodging of an embolus is formed in one part of the body and when it

breaks lose, travels along the bloodstream until it lodges in an artery or in a vessel of the brain. The third form of stroke is the lacunar infarction in which occurs an occlusion of arterioles, which are the very small end of arteries that penetrate into the brain. The hemorrhagic strokes are caused by holes in the wall of small blood vessels (intracerebral hemorrhage) or can be due an aneurysm or a vascular malformation where there is a rupture of the artery and the blood leaks to the space around the brain (subarachnoid hemorrhage). Despite the different possible causes described above there are others of unknown cause (Lawrence & Brass, 1992) (Chino et al., 1994) (Hossmann & Heiss, 2009) (Furie et al., 2011).

After stroke, a great number of patients will need therapy, which depends on the patient's needs and symptoms. The most common areas affected are motor function, (paralysis or weakness on contralateral side of the brain's lesion, change in muscle tone), loss of sensation or feeling, dysphagia, vision and communication difficulties, automatic function affected, cognition and emotional problems. The patient may neglect the affected side, which signs include for example, ignoring people or objects on the affected visual hemifield, or walking to the good side. This neglecting behaviour is mainly due to impaired vision, weakness of muscles and altered sensations and in persons who have the right brain damage seems to be more difficult to treat (Lawrence & Brass, 1992) (Chino et al., 1994) (Platz et al., 2000) (Shahid et al., 2010). Most of the patients can suffer from depression at early, medium, and late stages of stroke recovery (Hackett et al., 2005).

Thus, this range of symptoms can vary from person to person and a common way of characterizing stroke injury is by analyzing the side of the brain affected. The left hemisphere affected will result in paralysis of the right side of the body, speech and language deficits, slow behavior, memory problems related to language and right-side neglect (less common than left-side). Damage in the right hemisphere can produce left side paralysis of the body, spatial-perceptual problems, left-side neglect, impulsive behavior and memory related impairments (Lawrence & Brass, 1992) (Chino et al., 1994) (Platz et al., 2000) (Amengual et al., 2014).

Imaging studies after stroke have associated the functional recovery with the reorganization in the periinfarct (area that surrounds an infarct) and the surround cortical areas. On a cellular level two main regenerative events occur in the periinfart cortex: axons develop new connections and establish new projection patterns, and newly born immature neurons migrate into periinfarct cortex. These results show that the cellular

environment after stroke is not only death and destruction, but rather a longer evolving process of neuronal regeneration (Nudo, 2006) (Carmichael, 2006) (Murphy & Corbett, 2009).

Previous studies have shown that white matter condition can be apparently improved following stroke, due to an increase of the fractional anisotropy, a diffusion tensor imaging and derived measure of white matter microstructure. That changes occur not just in the stroke hemisphere but also in the contralesional hemisphere. This result complements previous demonstrations of functional plasticity and will influence the network measures of efficiency of communication. Regions of reduced connectivity in patients tended to cluster around the stroke locations, and have shown evidence for reduced communicability in patients in the contralesional hemisphere. These areas (e.g. caudate, planum polare, Heschl's gyrus) in the contralesional hemisphere are remote from the site of primary damage, but are functionally connected, directly or indirectly, with their homologues in the lesioned hemisphere. In addition to regions of reduced activity, was also found some areas of greater communicability in patients, such us, the left (lesioned) anterior inferior temporal gyrus and posterior cingulate gyrus and the right (contralesional) orbitofrontal cortex, anterior temporal fusiform cortex and posterior inferior temporal gyrus. One possible interpretation of these changes is that the increased connectivity reflects adaptive changes in white matter structure that have occurred secondary to the stroke (Crofts et al., 2011) (Zappasodi et al., 2014).

In stroke patients, the different mechanism implicit on the functional changes of the motor system can be understood by several published studies. Johansen-Berg *et al.* (2002) and Loubinoux *et al.* (2003) studies have reported a different activation of the motor system in chronic stroke patients compared with controls (Johansen-Berg et al., 2002) (Loubinoux et al., 2003). Functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies have discovered bilateral activations over the primary motor cortex and Weiller *et al.* (1993) also found similar patterns on premotor cortical areas. In the same study it was described that, in chronic stroke patients compared with controls, there are a greater activation of the nonprimary motor areas such as premotor areas, the supplementary motor area (SMA), and parietal and insular cortex during simple movement. It has been suggested that these recruitment of nonprimary motor areas might reflect functional compensation. However, the temporal dynamics are different in the chronic phase than during the acute phase of the stroke, so it always should

be taken into account the spatial rearrangement of brain functions (Amengual et al., 2014).

2.3 Electroencephalography

Following a focal stroke, there are multiple ways in which the structure and function of the brain may change. The region immediately surrounding a stroke undergoes a potentially reversible structural change and anterograde or retrograde degeneration of axons intersecting or connecting with a lesion may occur (Crofts et al., 2011).

So, when a stroke patient is admitted at the hospital, is important to make a fast and accurate diagnosis to start the treatment as soon as possible. The clinical history may often be incomplete or misleading; the patient can perform a computed tomography (CT) scan which is only valuable to exclude hemorrhages, masses or other lesions. Multiparametric studies, like resonance magnetic imaging, are informative but are expensive and generally are not available at the hospital. Another diagnostic technique that is inexpensive, widely available and despite giving different information than the imaging techniques, the EEG is the best technique to show brain alterations after acute ischemic stroke.

EEG can add value to multiparametric imaging studies and neurologic examination because it reflects the neuronal function in acute ischemic stroke, which is important to an early diagnosis, outcome prediction, clinical management and seizure detection. The Rankin Scale Grade has been widely used as a clinical outcome measure for patients who have suffered a stroke, although in patients with severe deficits, this scale is not so accurate than early EEG analysis. Progressive alterations in EEG morphology, amplitude and frequency correlate with severity and volume cerebral ischemia. However, there are a “window of reversibility” between the early appearance of EEG abnormalities and neuronal death (Jordan, 2004) (Amengual et al., 2014) (Zappasodi et al., 2014).

2.4 Frequency-specificity of brain oscillations

EEG is a test that measures the electrical activity of the brain by using electrodes on the scalp and records waveforms reflecting the cortical electrical activity. The waveforms are subdivided into bandwidths and the majority of the EEG used in clinical practice identifies four periodic rhythms: alpha, beta, delta and theta. These rhythms are

distinguished by their different morphology, frequency (Hz or cycles/second) and amplitude (μV). The frequency is negatively associated with their amplitude, which means that when the frequency increases, the amplitude decreases. The delta band designates activity with a frequency below 4Hz and it is known to occur in deep sleep. In awake adult, rhythmic delta activity is usually an abnormal signal. EEG activity in the frequency range of 4 to less than 8 Hz is called theta. Irregular low-amplitude theta activity is usual a feature of the normal adult EEG and in the awake state has greatest amplitude in the posterior temporal regions. The range of frequencies from 8 to 13 Hz is called the alpha band. It occurs during wakefulness over the posterior regions of the head, generally with maximum amplitudes over the occipital areas. It is best seen with the eyes closed and during physical relaxation and relative mental inactivity. It is blocked or attenuated by attention, especially visual and mental effort. There are many oscillations at alpha frequencies with different origins, reactivity and functional significance. Many EEG recordings show activity at alpha frequency that arises from central motor regions, often with a specific waveform and with a reactivity that differs from occipital alpha. This is called mu rhythm. Rhythm at 7-11Hz, composed of arch-shaped waves occurs over the central or centro-parietal regions of the scalp during wakefulness. Blocked or attenuated primarily by contralateral movement, thought of movement, readiness to move or tactile stimulation. Amplitudes varies but is mostly below 50μV (Arroyo et al., 1993).

Activities between 14 and 40Hz over the fronto-central regions of the head during wakefulness are in general defined as beta activity. Usually has an amplitude below 30μV. Beta activity increases with drowsiness or light sleep and some drugs (e.g. barbiturates and benzodiazepines) can increase the amplitude of beta activity (Cooper et al., 2005).

The amplitude of oscillations is proportional to the number of synchronously active neural elements, so, the alpha rhythm reflects a bigger number of interconnected neurons and therewith an increasing number of coherently activated neurons than beta rhythm, which have a slower amplitude and bigger frequency (Pfurtscheller & Lopes da Silva, 1999).

EEG activity has an excellent temporal resolution which helps to provide precious information about the neural dynamics among premotor and motor areas during motor tasks. So, this technique is valuable when an event-related potential (ERP) component is

expected, as in the case over the motor or sensory structures where we have neural generators of ERPs (Pfurtscheller & Lopes da Silva, 1999) (Amengual et al., 2014).

So, ERP characterizes the response of cortical neurons due to alterations in afferent activity, while ERD and ERS reflect modifications in the activity of local interactions between main neurons and interneurons that regulate the frequency components of the ongoing EEG. The former is phase-locked and the latter is often not phase-locked to the event (figure 5) (Kalcher & Pfurtscheller, 1995) (Pfurtscheller & Lopes da Silva, 1999).

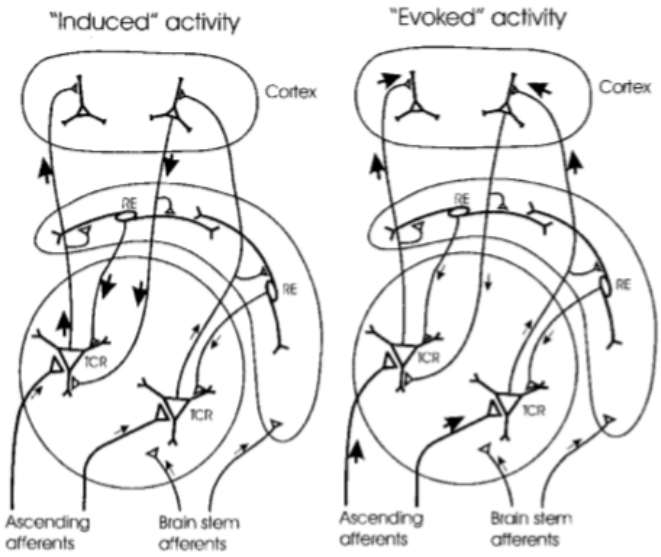


Figure 5. Schema for the generation of induced (ERD/ERS) and evoked (ERP) activity whereby the former is highly frequency-specific. TCR thalamic relay cells; RE reticular thalamic nucleus (Pfurtscheller & Lopes da Silva, 1999).

2.5 Characteristics of EEG patterns in stroke patients

The characteristic EEG pattern in mild cerebral ischemia shows a subtle decrease in the amplitude of fast activities (>13Hz). With increasing severity, in moderate to severe ischemia, the EEG pattern includes widespread polymorphic delta activity in the affected hemisphere maximally seen in frontotemporal and temporal regions, ipsilateral attenuation or loss of beta and alpha activity as well as sleep spindles, marked suppression of all higher EEG frequencies and contralateral frontal delta activity and intermittent projected bursts of delta activity. In vast subcortical acute ischemic stroke the EEG can express focal or generalized intermittent rhythmic theta and delta activity (Jordan, 2004) (Zappasodi et al., 2014).

Overall, the EEG predicts a poor outcome if continuous polymorphic delta with depression of alpha or beta activity in the affected hemisphere is found. Some authors describe that the degree of background depression independently correlated with outcome, on the other hand, others found that ipsilateral or contralateral background slowing on the initial EEG correlates with poor functional outcome. EEG predicted a good outcome by absence of slow activity with minimal decrease in background frequencies, or intermittent theta-delta activity with slight asymmetry of background activity (Jordan, 2004) (Zappasodi et al., 2014).

As it was described above, after stroke is common be affected the motor function. Chronic hemiplegia is a common long-term consequence of stroke, affecting 69% of stroke survivors. These deficits on motor function cause changes in neural activation of ipsilesional and contralesional hemisphere, during preparation and execution of movements performed with the affected side. In previous fMRI studies it was found that stroke patients during recovery had an increased ipsilesional activation and a decreased contralesional activation. Therefore, to understand the motor recovery process in stroke patients, it is important to study the neural mechanisms underlying brain plasticity and functional reorganization (Dean et al., 2012) (Amengual et al., 2014) (Tangwiriyasakul et al., 2014).

In the last decade, various novel stroke rehabilitative methods for motor recovery have been developed, which are based on the evidence of neuroplasticity. The methods which induce neuroplastic changes, lead to greater motor and functional recovery than traditional therapeutic approaches. New methods have been described for motor recovery such us motor imagery, constraint-induced movement therapy, robotic training, TMS and virtual training (Arya et al., 2011) (Najib et al., 2011).

2.6 Mu and beta synchronization and desynchronization in motor execution

The brain processes involved in generating and controlling movements through sensorimotor and associated cortical areas offers a window to how the information processing in multiple neuronal networks may be realized**.** This information can be study through oscillatory EEG signals where the components between 10 and 40 Hz have different patterns of spatiotemporal cerebral activation which reflects different neural mechanism related to movement (Pfurtscheller & Lopes da Silva, 1999) (Platz et al.,

2000) (Graimann et al. 2002) (Neuper et al., 2006) (Fu, 2006) (Takemi et al., 2013) (Rossiter et al., 2014).

The neural network that produces rhythmic EEG activity involves four elements: thalamic reticular nucleus (TRN) neurons, inhibitory local circuit neurons in thalamus, thalamocortical relay (TCR) neurons, and corticothalamic neurons. The TRN express $GABA_A$ receptors (ionotropic receptors and their ligand is γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (CNS)) and it has a key role in controlling the rhythmic activity. Not only the motor execution (ME), but also the motor imagery (MI) decreases mu and beta band recorded over the sensorimotor areas (designated as event-related desynchronization). ERD is considered to reflect a reduction in synchrony of the underlying neuronal populations. A possible mechanism for the generation of ERD during motor imaging is represented schematically in figure 6 (Takemi et al., 2013).

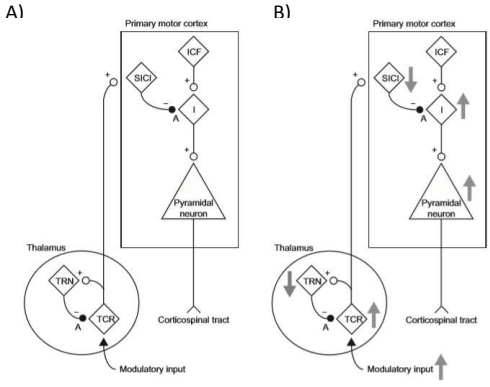


Figure 6. Diagram of the possible mechanism for the generation of ERD during motor imagery. A: rest condition. B: ERD during motor imagery. ERD during motor imagery induced a significant inhibition of $GABA_A$ transmission in both the thalamus and primary motor area and a significant facilitation of the excitatory modulatory input, the thalamocortical relay (TCR cells), the I wave-generating neurons, and the cortical pyramidal neurons. A, $GABA_A$ receptors; O, excitatory synapse; ●, inhibitory synapse; TRN, thalamic reticular nucleus neurons; I, group of I wave-generating neurons; short-interval intracortical inhibition and intracortical facilitation, neurons generating short-interval intracortical inhibition and intracortical facilitation, respectively; up and down arrows, increase and decrease in excitability, respectively (Takemi et al., 2013).

In the absence of sensory information or motor output, the alpha band usually arises at central areas. So, it has been considered that the mu rhythm (~10Hz) occurs by deactivated cortical areas and may represent a mechanism which reflects a cortical an iddling or inhibitory cortical activity. Preparation, execution of movement produces an ERD, about 2 seconds prior to the movement-onset, over the sensorimotor areas, in the mu rhythm and also in beta band (< 40 Hz) (Arroyo et al., 1993) (Pfurtscheller & Neuper, 1994) (Pfurtscheller et al., 1996) (Pfurtscheller et al., 1997) (Pfurtscheller & Lopes da Silva, 1999) (Platz et al., 2000) (Fu, 2006) (Takemi et al., 2013) (Rossiter et al., 2014).

The mu and beta ERD during motor preparation are more pronounced over the contralateral sensorimotor areas and then spread bilaterally with movement initiation. The topography of the alpha is different for the low alpha band (8–10 Hz) and the high alpha band (10–12 Hz). The lower alpha ERD reflects a widespread movement-type non-specific ERD and is more prominent at parietal electrodes and the topography of the higher alpha ERD is more similar to the central beta ERD (~20 Hz) and shows a more focused and movement-type specific pattern. It is of interest to notice that the localization of the higher alpha ERD is slightly more posterior compared to the beta ERD. This may be because mu rhythm is generated principally in the post-rolandic somatosensory area and the central beta rhythm in the pre-rolandic motor area. Therefore, what has been described shows that the motor execution is a combination of different processes and reflects different frequencies (i.e., 8-10, 10-12, 15-25 Hz) (Pfurtscheller et al., 1997) (Pfurtscheller & Lopes da Silva, 1999) (Pfurtscheller et al., 2000) (Platz et al., 2000) (Pineda, 2005) (Ilmoniemi & Kicic, 2010) (Ramos-Murguialday & Birbaumer, 2015).

The ERD of alpha band and beta frequencies is an electrophysiological activity associated with an activated cortical network, organized to process information with the increased excitability of cortical neurons. So, the pre-movement ERD can be due a readiness of the neural network in sensorimotor areas. Although, once the movement sequence was learned, and it is performed more “mechanically”, the ERD is reduced. These results suggest that ERD in primary sensorimotor areas increases in association with learning a new motor task and decreases after the task has been learned (Pfurtscheller & Lopes da Silva, 1999) (Platz et al., 2000).

Despite this desynchronization in specific cortical areas, in other locations not engaged in the task is accompanied by an increase of synchronization in the alpha band. The fact that ERD and ERS happen at the same moment, but in different scalp areas, was named “focal ERD/surround ERS”. This is more specific for the higher alpha. It has been understood to be due a cortical inhibition of networks which are not correlated in a certain specific task. For example, voluntary hand movement can result in a hand area ERD and simultaneously in a foot area ERS, and voluntary foot movement can result in an opposite pattern, as shown in figure 7 (Pfurtscheller & Neuper, 1994) (Pfurtscheller et al., 1996) (Pfurtscheller et al., 1997) (Pfurtscheller & Lopes da Silva, 1999) (Pfurtscheller et al., 2006) (Neuper et al., 2006) (Ramos-Murguialday & Birbaumer, 2015).

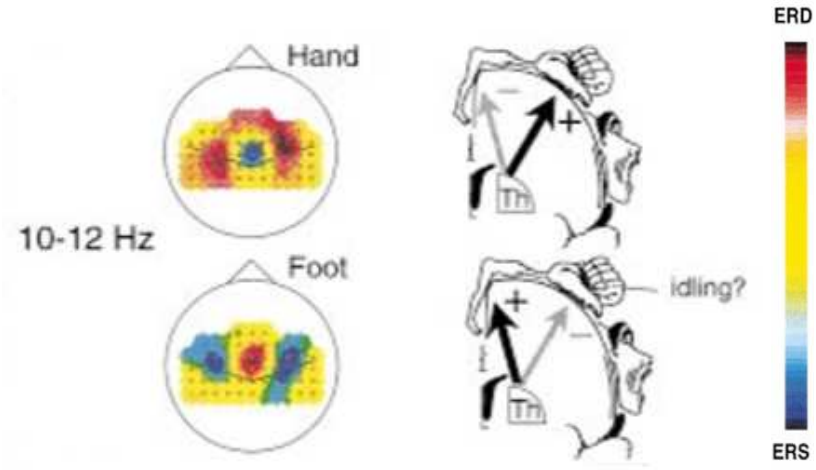


Figure 7. Maps displaying ERD and ERS during voluntary movement of the hand and movement of the foot. The motor homunculus represent a possible mechanism of cortical activation/deactivation gated by thalamic structures (Pfurtscheller & Lopes da Silva, 1999).

The overall finding for beta rhythm is that during preparation and execution of movement its oscillations are desynchronized. When movement ends, a robust phenomenon happens in the contralateral primary sensorimotor cortex. A focus of beta activity recuperates in less than one second, with a maximum around 1000ms, and is start to seeing a short-lasting beta burst. In the meanwhile, it is still seen the mu rhythm with a desynchronized pattern of low amplitude. The beta rebound activity is being described as high degree in somatotopical specificity for finger, arm and foot movement, see figure 8. In previous studies has been described that this beta synchronization, after the end of movement, describes a state of deactivation and consequently, a reduced level of excitability of the motor neurons (Pfurtscheller et al., 1997) (Pfurtscheller & Lopes da Silva, 1999) (Neuper & Pfurtscheller, 2001) (Neuper et al., 2006) (Rossiter et al., 2014).

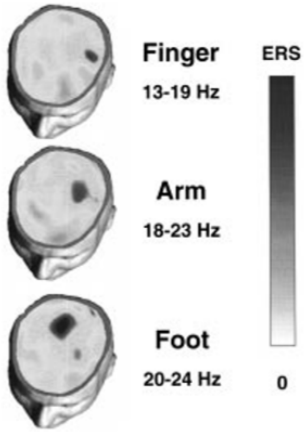


Figure 8. Movement-specific location of the beta ERS after finger, arm and foot movement. Note the different subject-specific frequency bands, lowest with finger and highest with arm and foot movement, respectively. `Black' indicates location of maximal ERS (Pfurtscheller & Lopes da Silva, 1999).

According to Park *et al.* (2014) an active movement induced larger ERD in the beta band than passive movement in bilateral sensorimotor cortical areas and the SMA. A larger ERD, associated with active movement, was observed when participants executed actively and passively two type of movements: grasping and supination (Park et al., 2014). The SMA area also displays rhythmic activity within the alpha band and, when a subject is preparing and planning a movement, mu rhythm is desynchronized (Pfurtscheller et al., 1997).

In the work of Rossiter et al. (2014) the movement-related beta desynchronization in contralateral primary motor cortex in chronic stroke patients was studied. They found the movement-related beta desynchronization in stroke patients with motor impairment to be markedly reduced compared with control subjects. They considered that impaired modulation of beta oscillations during affected hand grip is detrimental to motor control, highlighting this as a potential therapeutic target in neurorehabilitation.

A study of Fu *et al.* (2006) revealed the effects of hand dominance on pre-movement brain activity between control and chronic stroke patients. They showed that the effect of hand dominance on ERD is significantly higher when the non-dominant arm was tested versus the dominant arm. This conclusion showed that handedness has a major impact on the pre-movement brain activity in stroke survivors and highlights hand-dominance as an important independent variable in the design of future experiments on stroke survivors.

2.7 Mu and beta synchronization and desynchronization in motor imagery

Stroke patients with motor deficits need to do physical training which is the standard therapy for stroke rehabilitation, although some of them entirely lose their capability to move the affected limb. A new alternative therapy has been introduced: motor imagery. The MI, defined as the imagined rehearsal of motor act, is available to any stroke patient, with or without muscle activity, being only necessary voluntary mental activity.

This technique does not replace physical training, but can promote or accelerate stroke recovery. As it was described, to imagine a movement involves part of the network which is also activated in actual execution of that movement. The cortical neurons are

activated while subjects are imaging a movement, resulting in an ERD detected over the sensorimotor cortex (Scherer et al., 2007) (Shahid et al., 2010) (Cincotti et al., 2012) (Takemi et al., 2013) (Wright et al., 2014) (Park et al., 2014) (Tangwiriyasakul et al., 2014).

A study of the primary motor cortex while it generates MI, reveals that beta activity appears significantly involved in the internal representation of movements irrespective of whether the motor behavior is actually executed or just imagined (Schnitzler, Salenius, Salmelin, Jousmäki, & Hari, 1997). In other previous studies was shown that motor movement or imagery are associated to the same cortical areas, so the patterns of desynchronization for beta and mu band are similar. Although, in a study of Pfurtscheller *et al.* (1997) they found that imagination of movement (in contrast to execution of movement) did not show bilaterally symmetrical ERD patterns. In contrast, imagination activated a significant ipsilateral ERS in parallel with the contralateral ERD (Pfurtscheller & Lopes da Silva, 1999) (McFarland et al., 2000) (Wright et al., 2014).

According to Scherer *et al.* (2007), in hemiparetic stroke patients, the undamaged hand motor movement and MI activates the undamaged contralateral hemisphere, through desynchronization in the mu and beta band. The affected ipsilateral sensorimotor area does not show that activation pattern. ME and MI of the damaged hand produce very similar patterns in the unaffected hemisphere as found with unaffected hand MI. Due to the damage on the structures underlying the brain no common activation pattern was found on the affected hemisphere. Some studies have also shown that during movement execution or imagery of the affected hand there is an activation of homologous areas in the unaffected hemisphere. These studies suggested a potentially beneficial mechanism in which the healthy hemisphere compensated for the functional deficit arising from the lesion (Platz et al., 2000) (Murase et al., 2004) (Wiese et al., 2005) .

Kaiser *et al*. (2012) found that during MI of the affected hand, patients with higher impairment showed higher ERD in the contralesional hemisphere as compared with patients with less impairment. This higher contralesional activation may be related with poor recovery and higher degrees of stroke impairment. In addition, a significant relationship was identified between ipsilesional ERD during MI of the affected hand and the degree of spasticity. Stronger ERD in the unaffected hemisphere was associated with higher spasticity.

2.8 Motor execution versus motor imagery

In the study of McFarland *et al.* (2000) the differences of mu and beta rhythms, between movements and imagined movements for each hand in normal subjects were evaluated.

It was shown that left or right-hand movement results in a desynchronization of mu and beta rhythm which is higher on the contralateral side to the movement. The comparison movement versus rest shows that hemispheric asymmetries in the beta and mu rhythm are greater in the right-hand (dominant) than with left-hand movement. In motor imagery versus rest, the results are similar for movement versus rest, but reduced in magnitude. The desynchronization for motor imagery on the contralateral side, is more prominent than for movement. Hemispheric asymmetry is more marked in the right hand imagery for the mu rhythm, and for the beta band is more pronounced for left hand imagery. At CZ, central site, we have mainly beta desynchronization, independently if it is right or left movement or left or right-hand imagery (McFarland et al., 2000).

The study of topographies for movement (right and left) versus rest and imagery versus rest for mu rhythm, has shown two foci of desynchronization, one over sensorimotor cortex on each side. The focus are stronger on the left side of the brain and also for movement than motor imagery. For beta band, movement and imagery, shows a more diffuse desynchronization on the vertex and extends more to the left side (McFarland et al., 2000).

2.9 Transcranial Magnetic Stimulation

TMS is based on the principle of electromagnetic induction of an electric field in the brain, see figure 9. It provides, for the first time, a non-invasive, safe and painless method where it is possible to activate the human motor cortex and assess the integrity of the central motor pathways. It has a greater potential of neuromodulation for rehabilitation and therapy, and summated with repeated sessions its effects leads to an outlasting a stimulation session. TMS can also interfere with brain activity, so when TMS is combined with EEG, it provides useful information to assess cortical excitability and connectivity (Izumi et al., 1997) (Kobayashi & Pascual-Leone, 2003) (Thut & Pascual-Leone, 2010a) (Thut & Pascual-Leone, 2010b) (Ilmoniemi & Kicic, 2010) (Groppa et al., 2012) (Premoli et al., 2014).

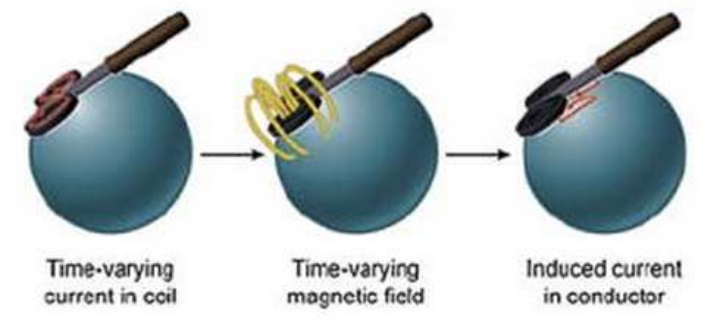


Figure 9. Example of a time-pulsed current when is discharged through the TMS coil. The resulting time-varying magnetic field is focused onto underlying neural tissue. The eddying currents, produced in the tissue, can affect the neural activity during and after stimulation (Najib et al., 2011).

Through a rapidly changed pulse current, a magnetic stimulating coil placed over a person's head can generate a strong magnetic field that can cross penetrate the scalp, causing a secondary induction current at adjacent nerve tissues. When TMS is applied to the motor cortex at a certain stimulation intensity, motor evoked potential (MEP) can be recorded at the contralateral extremity muscles. The amplitude of the MEP reflects not only the integrity of the corticospinal tract but also the excitability of motor cortex and nerve roots and the conduction along the peripheral motor pathway to the muscles. When a single-pulse stimulus is applied to the motor cortex, the motor threshold (MT) indicates the lowest TMS intensity necessary to evoke MEPs. It is necessary to define a motor threshold in which MEPs have more than 50 μV peak-to-peak amplitude in at least 50% of successive trials, when activating a target muscle. MEP is an electrical potential difference detected using bipolar surface electromyography (EMG) over the target muscle. The most common muscles which have been used for the studied of TMS are the intrinsic hand muscles (the first dorsal interosseous and abductor policies brevis muscles). Motor threshold is supposed to reflect membrane excitability of corticospinal neurons and interneurons projecting onto these neurons in the motor cortex, as well as the excitability of motor neurons in the spinal cord, neuromuscular junctions and muscle, see figure 10 and 11 (Kobayashi & Pascual-Leone, 2003) (Xie & Zhang, 2012) (Cortes et al., 2012) (Groppa et al., 2012).

In stroke patients the motor threshold and the silent period is often increased and the contralateral MEPs acutely after a stroke relate to a favorable recovery, while the absence of MEPs indicates a poor outcome. These changes may be attributed to some of the following: loss of neurons, altered membrane excitability in the remaining cells, increased cortical inhibition, compromised conduction, and dispersion of the excitatory

volleys onto motoneurons (Eliassen et al., 2008) (Kobayashi & Pascual-Leone, 2003) (Cortes et al., 2012).

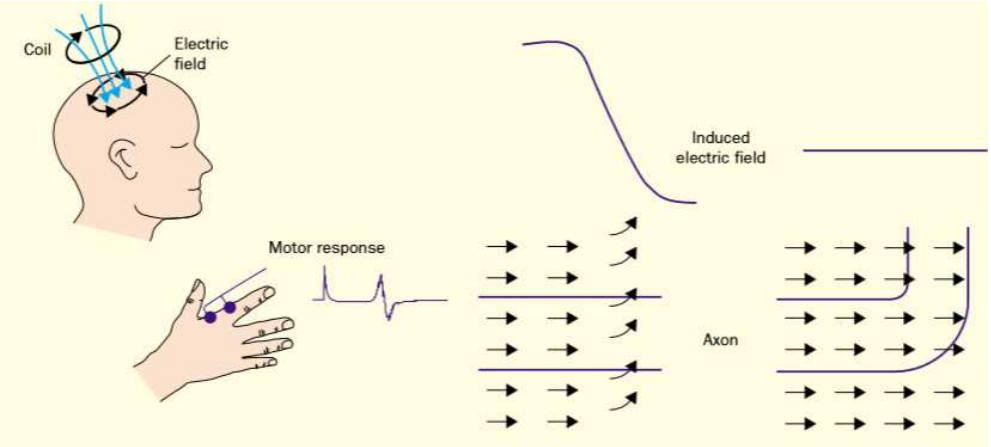


Figure 10. Principle of TMS. Left: the current flowing briefly in the coil generates a changing magnetic field that induces an electric current in the tissue, in the opposite direction. Middle: schematic illustration of the current flow due to the induced electric field that changes along the length of a nerve fiber and results in a transmembrane current. Right: a bent nerve and the uniform current in the uniform electric field also results in a transmembrane current (Kobayashi & Pascual-Leone, 2003).

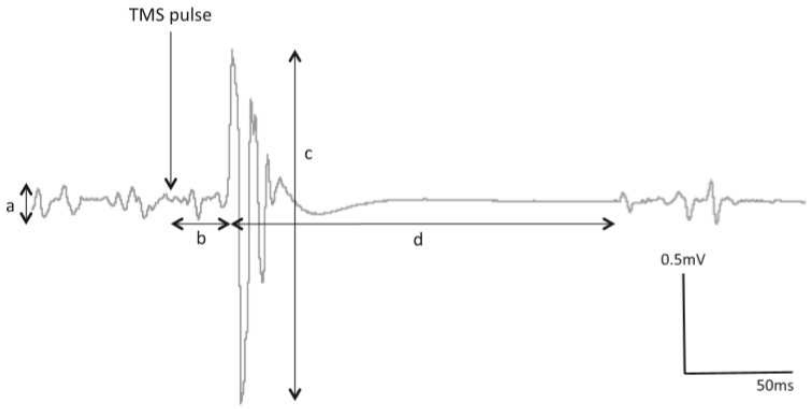


Figure 11. TMS-derived measures of cortical excitability. Schematic of motor-evoked potential characteristics, when a single pulse is recorded from a muscle with as light contraction. (A) background EMG; (B) latency; (C) peak-to-peak amplitude; (D) silent period (Cortes et al., 2012).

The TMS induces electric fields and depends on the relative location and orientation of the coil and the head, the head's large-scale structure and the local details of conductivity. These electric charge in the brain may depolarize pyramidal neurons located at the superficial cortical layers and therefore, voltage-sensitive ion channels are opened and action potentials are originated. The EEG records a linear projection of these synaptic activations. So, EEG signals can be used to quantify and to locate the postsynaptic current distribution (Kobayashi & Pascual-Leone, 2003) (Rossini & Rossi, 2007) (Ilmoniemi & Kicic, 2010) (Groppa et al., 2012).

The evoked responses on EEG from TMS are normally reproducible, because the delivery and targeting of TMS is well measured and constant from pulse to pulse and between experiments. After a single-pulse TMS in the motor cortex, several components of the EEG response can be identified: N15, P30, N45, P55, N100, P180. Although these components are not universal because the inter-individual differences, the coil location and orientation, state of the cortex and the vigilance of the subject, interfere with these components. An important feature of TMS-evoked EEG topography is that spreads from stimulation site ipsilaterally via association fibers and contralaterally via transcallosal fibers and to subcortical structures via projection fibers. So, when one cortical hemisphere was stimulated, an increased EEG activity can be seen in a number of adjacent electrodes, suggesting the spread of TMS-evoked activity to anatomically interconnected cortical areas (Izumi et al., 1997) (Ilmoniemi & Kicic, 2010).

TMS over the primary motor cortex elicits a sequence of TMS-evoked EEG potential which last for up to 300ms. There are two phases of inhibition after electrical stimulation of a cortical area: the first inhibition occurs at short latencies <50ms and the second inhibition has a delay onset and is long-lasting, 50-200ms. According to Premoli *el at.* (2014) the early inhibition represents activity of α1 subunit of $GABA_A$ (ionotropic receptor) receptors, whereas the N100 represents the activity of $GABA_B$ (metabotropic transmembrane receptors) receptors.

EEG coherence analysis exhibited that after stroke cortico–cortical connections were reduced in the stroke hemisphere. When TMS is applied, the mu and beta rhythm are also frequently affected, as well, the background activity at rest. So, TMS can alter the spectrum of the EEG signal. For example, recorded from adjacent electrodes TMS to primary motor area (M1) increases the power of the beta-frequency. On the other hand, the effect of M1 TMS on the alpha power increases with the intensity of TMS and the number of pulses administered. This effect is associated with the reduction in MEP size (Gerloff et al., 2006) (Thut & Pascual-Leone, 2010a) (Thut & Pascual-Leone, 2010b) (Ilmoniemi & Kicic, 2010) (Takemi et al., 2013).

TMS can be applied in three different ways: one stimulus at a time, single-pulse TMS; two pulses separated by a variable interval, paired-pulse TMS (pp-TMS); or in trains, repetitive TMS (rTMS). Single-pulse TMS is safe and valuable for investigating, however, and rTMS is a more powerful and potentially risky modality, capable of regionally blocking or facilitating cortical processes (Wassermann, 1996) (Rossi et al.,

2009) (Williams et al., 2010) (Cortes et al., 2012) (Groppa et al., 2012) (Takemi et al., 2013).

Single-pulse TMS is a useful tool for investigating various aspects of human neurophysiology, such as mapping motor cortical outputs, central motor conduction time, and causal chronometry in brain-behavior relations. In paired pulse techniques TMS stimulation can be delivered to a single cortical target using the same coil or to two different brain regions using two different coils. Paired-pulse TMS can be used to measure intracortical facilitation and inhibition, as well as study cortico–cortical interactions in both hemispheres. There are three main types of pp-TMS protocols where one aims to study the short-interval intracortical inhibition (SICI), other the long-interval intracortical inhibition (LICI) and the last the intracortical facilitation (ICF). The rTMS can stimulate with 'high-frequency' rTMS when stimulus rates of more than 1 Hz, and 'low-frequency' rTMS when stimulus rates of 1 Hz or less. Depending if rTMS is stimulating ≤1 or >1, these frequencies characterize different physiological effects and different risk degrees associated with low- and high frequency stimulation (Wassermann, 1996) (Rossi et al., 2009) (Williams et al., 2010) (Cortes et al., 2012) (Groppa et al., 2012) (Takemi et al., 2013).

The traditional repetitive stimulation protocols are known to have a large inter-individual variability in the effects produced. This variability depends, among other factors, on the frequency and duration of the stimulation. When it is applied conventional rTMS protocols the effects will range from 15 to 70 minutes and these effects do not differ between low and high frequency protocols. The effects of high hrequency TMS (1-20Hz) is increased by increasing the number of pulses and the number of trains. On the other hand, low frequency TMS (0.9-1Hz) shows a negative relationship between aftereffects and TMS-intensity, which have stronger suppressive effects with higher intensities. So, for example, the rTMS of 1Hz is necessary 30 minutes of stimulation to have an aftereffect for around 30 minutes (Di Lazzaro et al., 2005) (Thut & Pascual-Leone, 2010a) (Thut & Pascual-Leone, 2010b).

Recently, Huang *et al.* (2005) settled a "theta burst" paradigm to the human motor cortex using a short burst of low intensity (80% active motor threshold) at high-frequency (50Hz). The pulses are repeated at 5 Hz, which mimics the frequency of theta band in the EEG. The plasticity induced by theta-burst stimulation (TBS) shares properties with long term potention (LTP) and long term depression (LTD) mechanisms of synaptic efficacy,

but the precise mechanisms in humans are largely unknown. The TBS protocols are attractive because they are short lasting and low intensity stimulation is generally sufficient to induce robust, although reversible, physiological aftereffects. The delivery pattern of TBS (continuous TBS versus intermittent TBS) can also induce robust and long-lasting modulation of cortical excitability. The difference of these two patterns are on measurement of the excitability of the motor cortex, as monitored by the amplitude of MEPs, which can be increased or decreased. The cTBS decreases the amplitude of MEPs, while they are increased by intermittent TBS (iTBS) (Huang et al., 2005) (Di Lazzaro et al., 2005) (Ishikawa et al., 2007) (Goldsworthy et al., 2012) (Vernet et al., 2013).

When TBS is delivered over the hand representations of M1 projecting to a distal hand muscle has been shown to produce a larger and long-lasting inhibition compared with proximal hand muscles, which may indicates that intracortical networks are not similar across different motor representations (Martin et al., 2006).

TBS can facilitate M1 excitability when delivered intermittently or suppress M1 excitability when delivered continuously. In a study of Ishikawa *et al.* (2007) they showed that cTBS for 40 sec over M1 reduces the amplitude of MEPs for about 60 min after the end of the train. But, the new finding is that cTBS over M1 also suppressed MEPs evoked from the opposite M1.

2.10 TMS application after stroke

The great promise in the use of TMS in a clinical domain is the possibility for plastic reorganization of cortical circuits (Rossini & Rossi, 2007). Motor deficits in stroke patients is a consequence from the disturbance of the corticospinal tract and TMS studies have found that the level of corticospinal impairment is related to the clinical impairment. These damage of the corticospinal tract can be compensated by the activity in other regions of the motor system, such as, the contra-M1, SMA, and parietal area that are linked to a cortical level and can project directly to the motor neurons of the spinal cord. It was already described in previous studies with stroke patients an overactivation of the SMA and also other nonprimary motor regions such as the dorsolateral premotor cortex, ventrolateral motor cortex, cingulate motor areas, parietal cortex, and the insula (Platz et al., 2005) (Amengual et al., 2014). Remarkably, in normal participants, if they engage in more complex motor tasks these same regions of the extended motor system are recruited.

Which suggest that simple motor commands of the current task were more difficult for patients than controls (Amengual et al., 2014).

Stroke patients at the damaged hemisphere are affected not only by the infarct itself but also by the asymmetric inhibition from the unaffected hemisphere because there is a tendency for overactivation in the contralesional hemisphere soon after the stroke. Conceptually, rTMS has emerged as a potential tool to restore this interhemispheric dysbalance. In different studies the rTMS has been used in two ways: low-frequency stimulation (≤1 Hz) to the motor cortex of the unaffected hemisphere to reduce the excitability of the contralesional hemisphere or high-frequency stimulation (>1Hz) to the motor cortex of the lesioned hemisphere to increase excitability of the ipsilesional hemisphere (Hoyer & Celnik, 2011) (Conforto et al., 2012) (Corti et al., 2012) (Sung et al., 2013).

Recent studies have proved the safety of high frequency repetitive transcranial magnetic stimulation (HF-rTMS) in stroke patients, and it has been achieved good results. Although, low frequency repetitive transcranial magnetic stimulation (LF-rTMS) has also been shown promising results on motor deficits (Chang et al., 2010).

According to Conforto *et al.* (2012) hand motor impairment in stroke patients is caused by an excessive inhibition of the damaged hemisphere by the contralesional hemisphere. The results, showed that LF- rTMS to the contralesional motor cortex early after stroke is potentially effective to recover function of the paretic hand, in patients with mild to severe hemiparesis.

In a study of Higgings *et al.* (2013) LF- rTMS to the unaffected hemisphere was used in stroke patients. One of the evidences of this study suggests that an effective rTMS protocol is enough to induce an increase in cortical excitability of the lesioned hemisphere.

In other studies, it was evaluated HF-rTMS in acute stroke patients on motor recovery and on cortical excitability. The results confirm that HF-rTMS over motor cortex can enhance and maintain recovery and may be a useful add on therapy in treatment of acute stroke patients (Strens et al., 2003) (Kim et al., 2006) (Khedr et al., 2010).

Although, in a study where it is compared the long-term effect of five daily sessions of 1 versus 3 Hz rTMS on motor function in acute stroke it was found that LF-rTMS over the lesioned hemisphere can improve the recovery. After 3 months, the improvement was more marked in 1 Hz group (Khedr et al., 2010).

As it was described there are promising results for rTMS when they inhibited the unaffected hemisphere with low frequencies or when they stimulate the affected hemisphere with high frequencies (Sung et al., 2013). In this study we decided to use the TBS because is less short lasting than the other rTMS protocols and the aftereffects are longer. Between iTBS and cTBS there are studies that indicate that each one can be more efficient than the other in the recovery of stroke patients, and other studies indicate that both enhance the excitability of the lesioned motor cortex in stroke patients in acute phase, so are both efficacy (Di Lazzaro et al., 2008) (Hsu et al., 2012).

We decided to use only cTBS for safety reasons because the patients are in a sub-acute phase. The cTBS produce a significant decrease in cortico-spinal excitability, therefore it was applied on the unaffected hemisphere. According to previous studies already described we believe that cTBS will have promising results. So, below there are some studies with cTBS that support our idea.

According to Matsuda *et al.* (2013) when cTBS is applied to the non-affected side of M1 on the hand and shoulder area, it shows a potential tool for the recovery of the motor function on stroke patients. The mechanism of the enhancement is not only the recovery of the affected M1 but also spasticity modification, associated reaction and other factors. In another study, with more patients the results showed efficacy of cTBS on the restorative stage recovery in chronic stroke patients. The results have major importance for stroke rehabilitation, because the inhibitory effect of cTBS resulted in the improvement of the paretic arm movement (Manji et al., 2013).

Other study used the Wolf Motor Function Test to demonstrate the feasibility and efficacy of cTBS in improving the motor learning post-stroke. When cTBS is applied over M1 it was shown a large decrease in movement time compared by control stimulation (Meehan et al., 2011).

We only found three articles that used cTBS in stroke patients and none of them used EEG. So, it is important to have the EEG before and after cTBS to describe and understand the neurophysiologic effects of this protocol on primary motor cortex in stroke patients.

Combination of rTMS with EEG is a promising methodology to directly characterize brain responses at the cortical level and may thus provide a useful method to further characterize the neurophysiologic substrate of cTBS induced plasticity and enable assessment of cortical plasticity in regions outside the motor cortex. A previous study

with healthy participants, shown that cTBS increased the power in the theta band of eyes-closed resting EEG, whereas it decreased single-pulse TMS induced power in the theta and alpha bands. In addition, cTBS decreased the power in the beta band of eyes-closed resting EEG, whereas it increased single-pulse TMS-induced power in the beta band (Vernet et al., 2013). Another study used the EEG before and after cTBS have stimulated the primary motor cortex in healthy subjects. They found widespread reductions in functional connectivity in the alpha band and at the same time increased the functional connectivity in the high-beta bands, particularly between anterior and interhemispheric connections (Shafi et al., 2014).

3. OBJECTIVES

We considered that it is very important to consider manipulation of plasticity during the acute phase because most individual features with prognostic value appear in the first week after the stroke onset. This may help to better understand the pathophysiology of post-stroke recovery. Assessing brain activity during this phase will make us able to understand the mechanisms underlying brain plasticity and recognize its possible changes after stroke.

The EEG in stroke patients may reflect the global dysfunction of the motor system in the acute phase. Not only electrophysiological impairments reflect the functional state of neurons surviving cerebral ischemia, but their ability in providing recovery prognosis has been proved to be valuable. On TMS research, the HF, LF, cTBS and iTBS protocols have been shown to be able to improve motor function in stroke patients. We chose a TBS protocol because it has a shorter duration and its effects are more long-lasting than the other repetitive protocols. These two main reasons are important due to the conditions of the patients and the tests performed after TMS session to assess the effects of TBS last approximately one hour. We chose an inhibitory protocol, cTBS, for safety reasons. Also, although cTBS has demonstrated promising results in the literature, it is recent and, this way, there were not found many publications in this context.

This thesis is focused on the role of EEG and on the analysis of the functional reorganization of the motor system in stroke patients, before and after TMS. The main two goals are to understand how the healthy subjects and the stroke patients respond physiologically to the inhibitory protocol; and the second goal is to find if the hemispheric dominance influences the effect of cTBS protocol.

Main Objective

- Evaluate the physiological effects in healthy subjects and stroke patients induced by the cTBS protocol;
- Analyze if the hemispheric dominance influences the effect of cTBS protocol.

Secondary Objectives

- Compare the results between controls and stroke patients to analyze the main differences in the brain's physiology between subjects;

- Understand if cTBS can induce functionally meaningful alterations in the mu and beta rhythm after TMS, correlating to the physiological state of the brain before TMS;
- Analyze how the type of movement with hands and arms modulates changes in the mu and beta rhythms.

To pursue the thesis' goals, we had to perform different tasks that are listed in the Gantt chart (Figure 12).

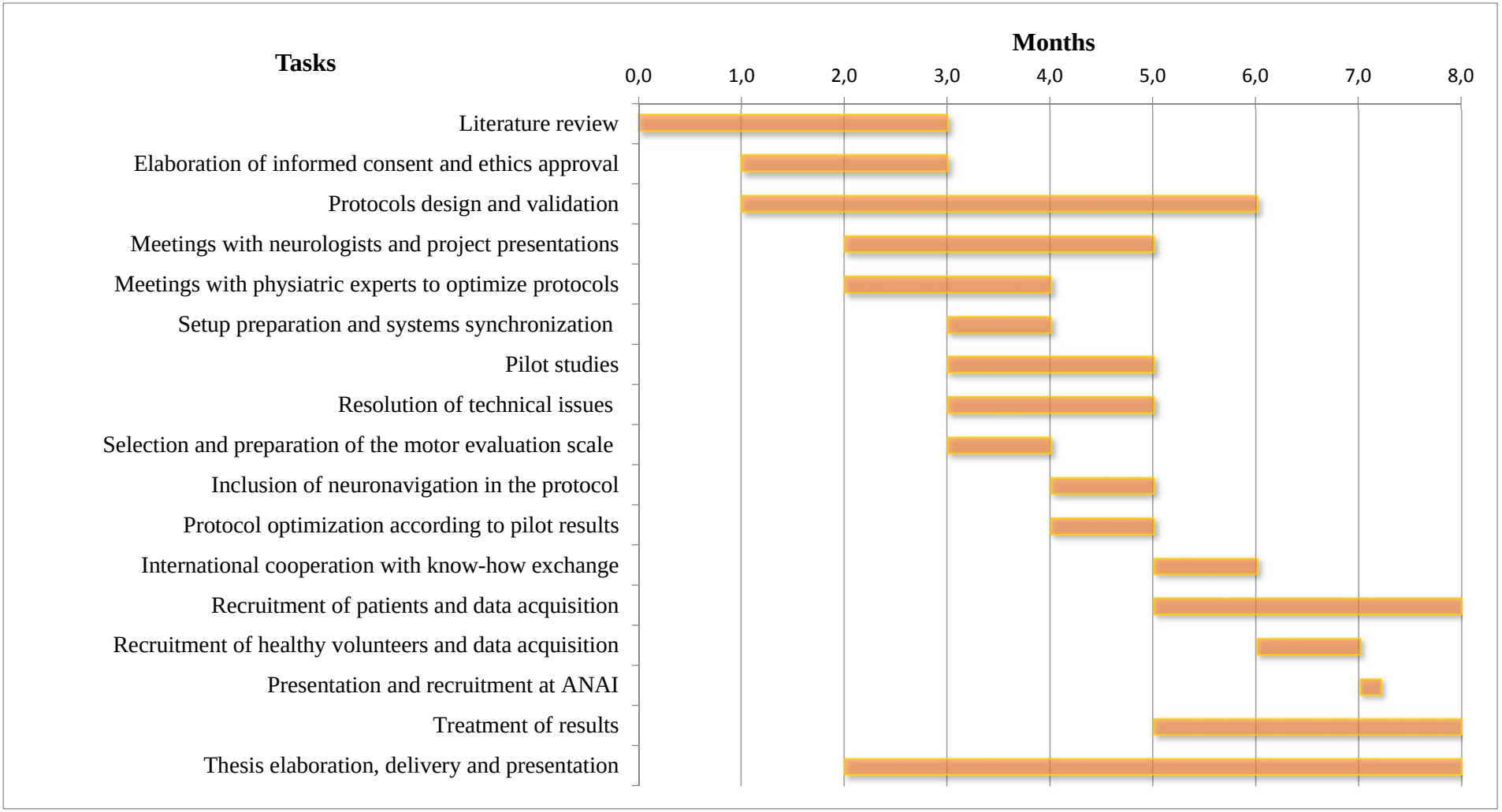


Figure 12. Scheduling of tasks

4. METHODOLOGY

The research project was carried out at ICNAS, guided by the group of Professor Miguel Castelo-Branco and with the collaboration of the Stroke unit of the Coimbra Hospital and University Center.

When the patients are admitted at CHUC they performed National Institutes of Health Stroke Scale (NIHSS) and the Modified Rankin Scale (MRS) to assess severity and functional ability. Both these scales have been frequently used in stroke patients, where the NIHSS is important to quantify the neurologic deficits most often seen in acute stroke patients (levels of consciousness, language, neglect, visual-field loss, extraocular movement, motor strength, ataxia, dysarthria, and sensory loss) and the MRS is used to measure the level of disability or dependency in the daily activities before the stroke occurred (Spilker et al., 1997; Wilson et al., 2002). The patients also performed a CT scan to assess and characterize the stroke lesion. All this information was stored in the clinical files. At the hospital, there were two clinical files; one for patients admitted in the study where the responsible doctor completed the relevant medical information, see appendix I, and, the other, for patients that did not join the study, appendix II. The patients who were admitted to the study, five to nine days after stroke the patients were sent to ICNAS. The procedures were carefully supervised by a neurologist and a nurse. For each stroke patient and control subject, a clinical report form at ICNAS was filled, see respectively, appendix III and IV. The study was approved by the institutional ethics review board and performed in accordance with Declaration of Helsinki.

Patients

The stroke patients were eligible for enrollment as study participants if they fulfilled all of the following inclusion criteria: (1) aged between 18 and 80 years, (2) poststroke period 7 ± 2 days, (3) first-ever middle cerebral artery stroke, (4) ischemic stroke, (5) cortico-subcortical lesion, (6) upper limb motor deficits, (7) ability to understand the tasks, (8) modified rankin scale pre- stroke ≤ 1. Patients who meet any of these criteria were not eligible for enrollment as study participants: (1) cognitive impairment, (2) previously documented dementia, (3) history of epilepsy, (4) neglect, (5) posterior or global aphasia, (6) artificial cochlear implant, (7) implanted pacemakers or medication pump, (8) pregnancy (9) drug and alcohol abuse and (10) intracranial metallic implant. One male, Caucasian, with 67 years old was recruited at CHUC.

The stroke patient was right-handed and he was assessed using the Edinburgh Handedness Inventory. The subject gave his written informed consent. Table 1 provides additional demographic data for the patient and a brain image obtained by Magnetic Resonance Imaging (MRI) is presented on figure 13.

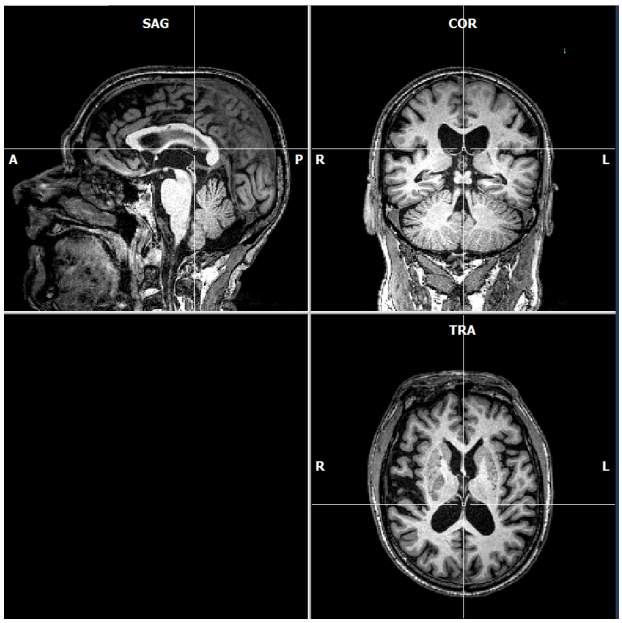


Figure 13. The lenticulostriate in the right hemisphere shows one of the earliest signs (and typical) of a stroke in acute/subacute phase: loss of differentiation between white matter and gray matter.

Controls

The subjects were eligible for enrollment as study participants if they fulfilled all of the following inclusion criteria: (1) aged between 18 and 80 years, (2) never had a stroke, (3) no motor deficits, (4) ability to understand the tasks. The exclusion criteria's were the same used for stroke patients.

Eleven Caucasian healthy subjects (4 man and 7 women), average age was 68,4 ± 4,2 years old (mean ± SD) were recruited. The 11 subjects were right-handed and were also assessed using the Edinburgh Handedness Inventory. Six subjects (2 man and 4 women) were stimulated in the left hemisphere and five subjects (2 man and 3 women) were stimulated in the right hemisphere. All subjects gave their written informed consent. Table 2 provides additional demographic data for the patient.

The subject number 11 has no relevant medical history and did not take any medication, was stimulated in the same hemisphere as the patient and her participation was very cooperative. Therefore, this subject will be used to representatively pair the results with the patient.

Patient	Sex	Age (years)	Lesion Location	Paretic Member	Handedness	Disease Course (days)	MRS	NIHSS
1	M	67	Right MCA	Left superior and inferior member	Right-handed	7	0	4

Table 1. Clinical features for each individual patient (MCA= Middle Cerebral Artery; MRS=Modified Rankin Scale; NIHSS= National Institutes of Health Stroke Scale)

Control	Sex	Age (years)	Clinical History	Medication	Handedness	Hemisphere Stimulated
1	M	66	Angina and cholesterol	Simvastatin and clopidogrel	Right-handed	Left hemisphere
2	F	61	High blood pressure and cholesterol	Moduretic and simvastatin	Right-handed	Right hemisphere
3	M	68	Tinnitus and benign prostatic hyperplasia	Tamsulosin	Right-handed	Left hemisphere
4	M	74	Cancer and tinnitus	Losartan and concor	Right-handed	Right hemisphere
5	F	68	Cholesterol	Tirox and Medipax	Right-handed	Left hemisphere
6	M	75	Benign prostatic hyperplasia and high blood pressure	Acetylsalicylic acid, amlodipine and tamsulosin	Right-handed	Right hemisphere
7	F	65	Poor circulation	Daflon and glucosamine	Right-handed	Left hemisphere
8	F	73	Vertiginous syndrome, high blood pressure, cholesterol and glaucoma	Simvastatin, amlodipine and timolol	Right-handed	Right hemisphere
9	F	68	High blood pressure and cholesterol.	Perindopril, simvastatin, concor and acetylsalicylic acid.	Right-handed	Left hemisphere
10	F	67	Hypothyroidism, cholesterol and high blood pressure.	Letter, simvastatin, aldactone and isoptin.	Right-handed	Right hemisphere
11	F	67	Nothing Relevant	Nothing	Right-handed	Left hemisphere

Table 2. Clinical features for each control

Blinding

Patients and controls were not aware of group assignment. To ensure anonymity, information about randomization and cTBS procedures both printed and electronic formats were locked in a cabinet, accessed only by researchers who perform cTBS. Patients did not discuss their experience during cTBS with therapists, or among each other. During cTBS the subjects received sham noise to not be influenced by any auditory stimulus.

Admission in the study

At CHUC, the neurologist and the nurses, two days per week, informed us, if there were patients that could be included in our study. If we had a patient, two days before or in the previous day of the experimental session, we went to the hospital with a neurologist to speak with the patient. We clarified the goals of the project, any questions the patient may had, and whenever necessary, we also spoke with his/her family. In the same day the patient filled out the following documents: informed consent, Edinburgh Handedness Scale, TMS security questionnaire and a MRI security questionnaire, see appendix V to VII. The responsible physician filled the clinical report form, appendix I, where the patient's medication was carefully analyzed, to ensure that the patient's safety was not compromised during the experimental procedure.

For control subjects, they were contacted through the ICNAS database. All the experimental procedure was explained and we used the same security measures in relation to the medication they were taking and their medical history.

Magnetic resonance imaging

Initially the patient underwent a MRI to generate a high-resolution, anatomical brain image to guide the TMS (MagPro X100, Magventure) using the Zebris Neuronavigation system. A 3-Tesla scanner (Magentom Trio, Tim System, Siemens) was used for acquisition of T1 images.

Experimental session

Participants were seated in a comfortable chair and all the experimental procedure lasted approximately 4 hours for stroke patient and around 3 hours for control subjects. The acquisition lab is seen on appendix VIII.

Wolf Motor Function Test

Before and after TMS only the stroke patient made a test to evaluate the affected upper limb, the Wolf Motor Function Test (WMFT). This test was performed by a neurologist and aimed to assess the motor function post stroke through the use of timed and functional tasks. The WMFT contains 17 tasks and it is composed of three parts: time, functional ability and strength; the strength items, 7 and 14, were not included in this study. The performance time of each timed task is documented and the calculation of performance time of 15 times tasks were calculated as the total time. When the task is not accomplished within 120 seconds, the performance time of the task is recorded as 120 seconds. (Morris et al., 2001). The functional ability scale (FAS) evaluates with a scale between 0 and 5, the quality of movement. Where 0 is when the patient does not attempt to move the arm being tested and 5 is when the arm performs the movement and seems to be normal (Pereira et al., 2011). The data form is on appendix IX and the template and the material necessary to perform the WMFT is represented on appendix X.

This test was used before and after cTBS to analyze if clinically there were any differences in the motor deficits of the affected limb in stroke patient. After 3 months the WMFT would be performed again to compare the clinical evaluation after the experimental procedure on the affected limb.

Electroencephalography recording and processing

The EEG data were recorded before and after TMS in stroke and healthy subjects. Movement-related potentials were recorded using a multichannel EEG device (SynAmps2 RT amplifier and Scan 4.5 software, Compumedics). We used an electrode cap fitted with 64-channel where each electrode was filled with a conductive paste, with ECI electro-gel. The low-pass filter was set at 200Hz, the high pass filter was set for direct current, and the acquisition sample rate used was 1000Hz. The notch filter was off during acquisition. The electrodes were positioned according to the international 10-10system at the sites Fp1, Fp2, Fpz, AF7. AF8, AF3, AF4, F1 to F8, Fz, FC1 to FC6, FCZ, FT7 to FT8, FT10, C1 to C6, CZ, T7 to T8, CP1 to CP6, CPz, TP7 to TP10, P1 to P8, Pz, PO3, PO4, PO7, PO8, POz, O1, O2 and Oz. An electrode placed between Cz and Cpz served as a reference, and between Fz and Fpz served as ground. Skin preparation gel with Nuprep and alcohol at 96% resulted in electrode impedances below 10 KΩ. All the EEG material used is represented on appendix XI.

Sequence of Motor Paradigm

First, 3 minutes of brain activity were recorded at rest to evaluate the physiological state, alternating between open and closed eyes.

Then, to analyze the electrophysiological biomarkers (e.g. mu rhythm, beta activity) the subject would perform for the upper limbs two different types of movement (first each limb individually and then simultaneously): arm elevation (upward, hold and downward) and thumb finger opposition. The task consisted in six repetitions of 15 seconds for each move, with an interval before, between and after repetitions of 15 seconds. Between each block of movements was an interval of 1 minute. The schematic of experimental design in functional imaging experiments is represented in table 3 and 4.

Conditions **Periods** **(15seconds)**	**Rest**	**Arm Elevation** **Upward Hold** **Downward**	**Rest**	**Repeat 6 times**
Right Arm Elevation				
	Lateral View	Lateral View	Lateral View	
Left Arm Elevation				
	Lateral View	Lateral View	Lateral View	
Both Arms Elevations				
	Lateral View	Lateral View	Lateral View	

Table 3. Task 1- Arm Elevation

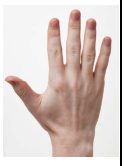
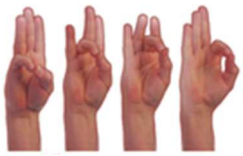
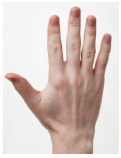
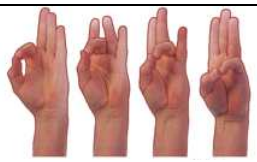
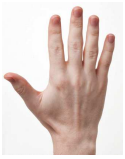
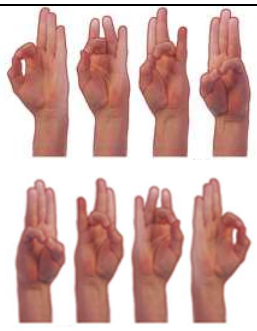
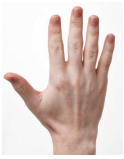
Conditions / Periods (15seconds)	Rest	Finger Opposition test	Rest	Repeat 6 times
Right Hand				
	Frontal View	Frontal View	Frontal View	
Left Hand				
	Frontal View	Frontal View	Frontal View	
Both Hands	 		 	
	Frontal View	Frontal View	Frontal View	

Table 4. Task 2- Finger Opposition Test (Incorporated, 2014)

Detailed Task Description

The recording of the brain activity and the sequence of motor paradigm were performed before and after cTBS to analyze the differences between both conditions. During 3 minutes the subject alternated between eyes open and eyes closed, each trial lasting 10 seconds. The motor tasks were each 9 minutes of arm elevation alternated with rest and 9 minutes of thumb finger opposition also alternated with rest. The interval between the two tasks was 1 minute. So, the total time considering the rest and the tasks was 22 minutes. This was repeated after cTBS, so the total time during the session was 44 minutes.

Before beginning the motor task it was explained and demonstrated to each subject the sequence of the motor paradigm. The signal "Go" was given to the subject to initiate the movement, and the "Stop" to stop the movement.

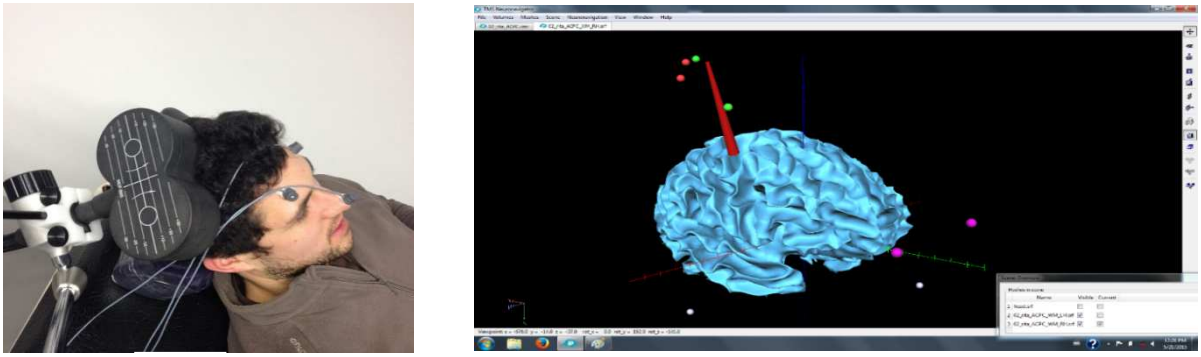
The sequence of each cycle of the motor paradigm was composed of thirteen blocks, from which seven were resting periods (1st, 3rd, 5th, 7th, 9th, 11th and 13rd block). The rest condition was used as reference. The experimental conditions took place in between these blocks. Each block had a duration of 15 seconds. The first condition was the arm elevation. The first cycle of the motor paradigm was first in the right arm, then the left arm and finally, both arms. Between the different arms we had an interval of 15 seconds.

Between the first and the second motor task an interval of 1 minute was defined. The second task was the finger opposition test and consisted in moving the thumb finger touching in other fingers sequentially. The cycle of the motor paradigm was the same used for the arm task.

Neuronavigation

During the session with stroke patient, the Zebris Neuronavigation System was used. The MRI images were used to create a head mesh reconstruction to ensure a reproducible and reliable coil placement when it were stimulating the M1 area, first for the affected hemisphere, and then for the unaffected hemisphere. The equipment that was used is represented on the appendix XII.

First, the Zebris Neuronavigation System, transformed the anatomical 3D files (*VMR data) in a DICOM extension. Then, we found the anterior commissure (AC) and the posterior commisure (PC) plane manually, to transform the VMR data into AC-PC plane. In the BrainVoyager QX software, we created the reconstructed head mesh. With the head mesh it was possible to do the real-time neuronavigation to an anatomical target site through the Neuronavigation System (see figure 14).



(A) (B)

Figure 14. (A) TMS coil above M1 area. (B) Lateral view - Brain meshes with the show pointer indicating the stimulation target site for the right hemisphere.

The optimal scalp location, over M1, for TMS-induced activation of the hand muscle was determined as the scalp location from which TMS induced MEPs of maximum peak-to-peak amplitude in the target muscle. Once the optimal spot was identified with the TMS coil, the brain location area was filled in the neuronavigation system, to guarantee a consistent coil placement at the optimal spot, for when we go back to the affected hemisphere and for 3 months later for follow-up evaluation.

Electromyography

For MEPs' measurement, surface EMG was recorded through the Ag/AgCl electrodes, using Ten20 conductive paste, see appendix XI. The active electrode was placed over the first dorsal interosseus muscle (FDI), the reference electrode over the metacarpophalangeal joint and the ground electrode over the wrist. When it was not possible to stimulate the FDI muscle, the electrodes were moved to the target muscle (figure 15). The EMG signal was acquired with a 1000 gain, filtered between 1–500 Hz, and the system that was used was the Acknowledge 4.1.

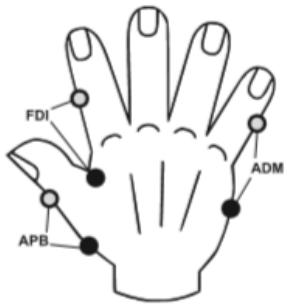


Figure 15. EMG electrode configuration - EMG recordings were derived from the FDI, abductor pollicis brevis (APB), and abductor digiti minimi (ADM) muscles using surface electrodes in bipolar belly-tendon montages (belly: dark gray; tendon: light grey) (Bergmann et al., 2009)

Data analysis MEPs' peak-to-peak amplitude was determined automatically using the Acknowledge 4.1 software, but checked trial-by-trial by visual inspection. For each subject, pre-cTBS MEPs' amplitude was defined around 1mV. After c-TBS we used the same intensity to compare the MEPs' amplitude before and after in both hemispheres.

Transcranial Magnetic Stimulation

TMS was applied with a figure-of-eight-shaped coil (outer diameter of each coil: approximately 7 cm) connected to a MagPro X100m magnetic stimulator (Magstim, Farum, Denmark). The coil was positioned tangentially to the scalp above M1 area with the handle pointing backward and laterally at an angle of about 45° to the sagittal plane.

All participants wore earplugs during TMS to protect them from possible acoustic trauma, and reduce contamination of TMS-evoked potentials by auditory responses to the clicks produced by the discharge of the TMS coil, see appendix XIII. According to Rossi *et al*. (2009) there is a list of drugs that can increase the risk for a seizure. So, for each stroke patient and healthy subject it was verified if they were taken any drugs that were on that list.

The control subjects received the cTBS protocol alternating between the dominant or non-dominant hemisphere and the subjects were randomized (1:1). The stroke patient were also randomized in a ratio 1:1 and they were divided in two groups: one group receives real stimulation and the other placebo. In both groups the hemisphere that receives the real or sham stimulation was always the non-affected hemisphere.

Initially, for stroke patient, we started with the affected hemisphere where we found the optimal coil position over the primary motor area. The control subjects started on the opposite hemisphere to the one that received the cTBS protocol. The optimal site of stimulation on the skull was defined as the location where the largest MEPs in the muscle of the upper limb was elicited on surface electromyography. The motor threshold of the muscle of the upper limb was defined as the intensity of stimulation output intensity capable of inducing a visible muscle twitching MEP. The rest motor threshold (rMT) was defined as the intensity of stimulation output intensity capable of inducing a MEP with 1mV peak-to-peak amplitude, in relaxed muscles in at least 5 of the 10 trials. The pp-TMS was performed before and after cTBS and it was used to measure cortical excitability on the hemisphere. Paired-pulse TMS protocols that were used to investigate were SICI, LICI and ICF. For ICF and SICI protocols subthreshold conditioning stimuli were set at 80% of the rMT and prior to the suprathreshold test stimulus adjusted to 120% of the rMT. For LICI protocol both threshold stimuli were 100% of the rMT. To estabilish a pre-cTBS baseline measure, in each protocol 10 MEPs' were recorded, where for the ICF and SICI were set at 120% of the rMT and for the LICI it was set at 100% of the rMT. The pulses were delivered randomly with an interstimulous intervals around 1, 3 and 5ms for the ICI protocol. The ICF used an interstimulous intervals for 10, 15, and 20ms. The last protocol, LICI, the interstimulous intervals were 50, 100 and 150ms. For the three protocols the mean delay was 9ms.

Then, for all subjects we went to the contralateral hemisphere where first we found the motor threshold and then, the rMT. The rMT induced a MEP with 1mV peak-to-peak

amplitude, in relaxed muscles, and then it was verified if was reproducible in at least 5 of the 10 trials. After finding all motor thresholds, we did 20 MEPs' pulses at 100% of the rMT to achieve our baseline. Then, we found the active motor threshold (AMT), asking to the subject to elevate both arms, and search for the lowest intensity that was able to put the hand muscle twitching. The intensity was fixed at 80% of AMT to do cTBS. The cTBS was applied with parameters similar to those used by Huang *et al.* (2005): three pulses at 50 Hz, with an interval of 200ms between the last pulse of a triplet and the first pulse of a triplet, for a total number of 600 pulses. After the cTBS protocol we waited 5 minutes to achieve the maximum effects of this inhibitory protocol to perform all tests in the time-window. Then, we repeated the 20 MEPs' pulses at 100% with the same intensity of the rMT that was found pre-cTBS.

Finally, we went back to the initial hemisphere to repeat the three protocols pp-pulse. A scheme of all experimental procedure for stroke patient and control subjects is represented on appendix XIV and on appendix XV, respectively.

Follow-up

A follow-up is going to be performed only for stroke patient three months later after this experimental procedure. The stroke patient will repeat the NIHSS and MRS at the hospital. Then, the patient is going to ICNAS to perform the WMFT, the EEG and the pp-TMS in both hemispheres. The neuronavigation system saved the spot for both hemispheres, which is important to ensure an evaluation in the same place within 3 months.

EEG Data Analysis

EEG data recorded were processed offline using the Scan 4.5 software and EEGlab toolbox running in a MATLAB environment (Mathworks). The recorded EEG signals were filtered between 1-45Hz to remove the artefacts using Scan Edit 4.5. Using the EEGlab toolbox the filtered EEG were down-sampled from 1000 Hz to 250 Hz. It was removed the EMG, HEO, VEO and EKG channels. When we had channels with bad EEG signal we applied the spherical interpolation. The muscle artefacts were removed by visual inspection and ICA was run to remove eyes movement and blink. The EEG signals were analyzed with average reference (figure 16). The continuous datasets were recorded in a single session and it was important to separate into epochs defining different task

conditions. So, during EEG recording we used different events to assess eyes open and closed, and the movement for the right arm/hand, left arm/hand and both arm/hand for the movement onset, offset and the rest period. The data epoch's time locked to events of interest were extracted from the continuous data from 2000ms before to 10000ms after for eyes closed or open. The epochs defined for motor tasks were -8000ms to -2000ms to define our baseline before movement, -2000ms to 4000ms when the subject is beginning the movement, 4000ms to 10000ms during motor task and -2000 to 4000ms after movement (see figure 17).

According to Tangwiriyasakul *et al.* (2013) it is important to have a specific baseline before the cTBS and after the cTBS protocol because the baseline can affect the ERD. For this reason we have a baseline (rest period) before the inhibitory protocol and other baseline (rest period) after the cTBS to assess if the brain activity is changed and to analyze ERD and ERS of mu and beta band.

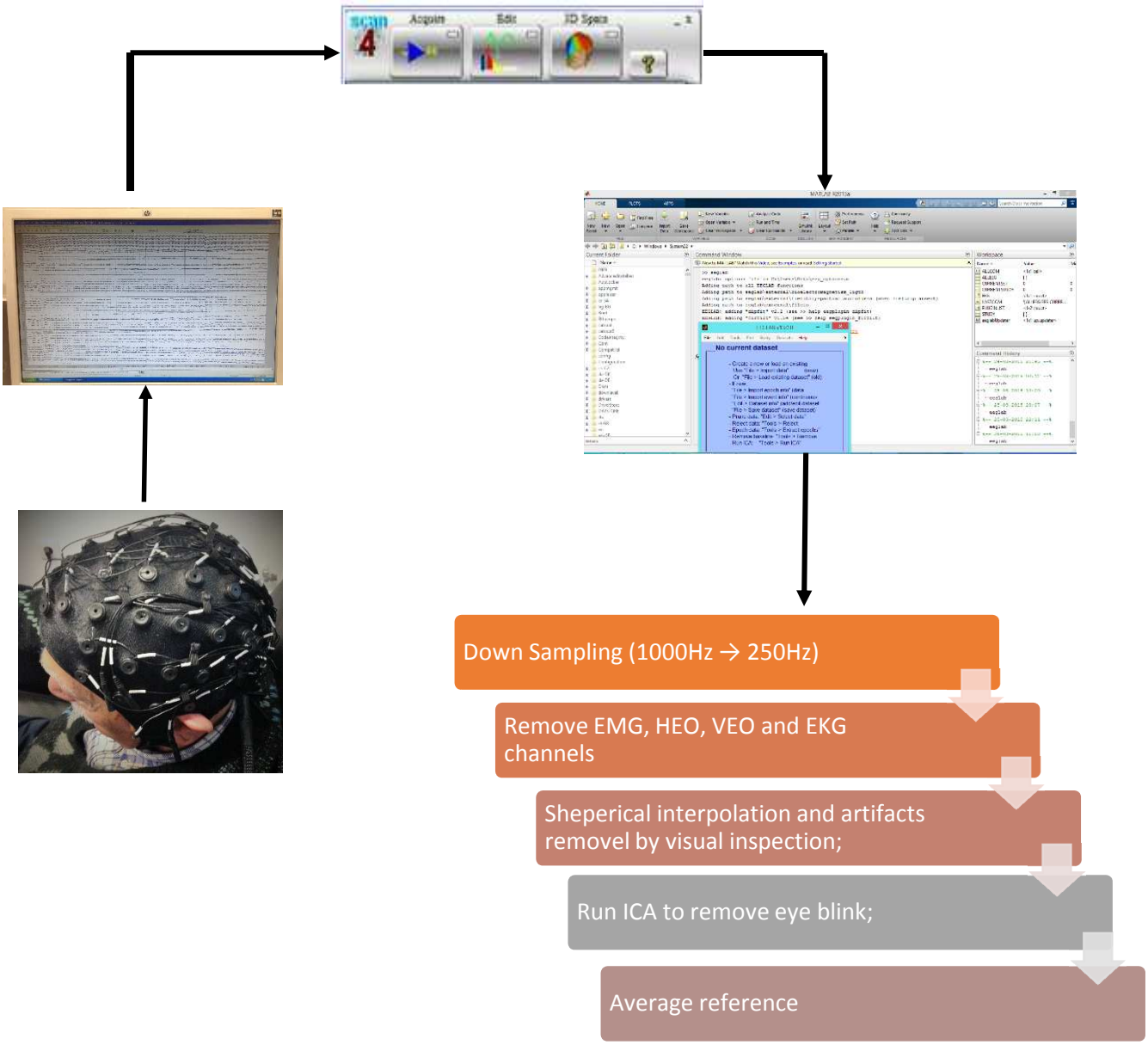


Figure 16. EEG cap acquires the signal from the brain and it is possible to see the recording in the computer through Scan 4.5 software. The recorded EEG is filtered in the Scan Edit 4.5. The scheme represents the EEG preprocessing procedure using the EEGLAB Matllab toolbox.

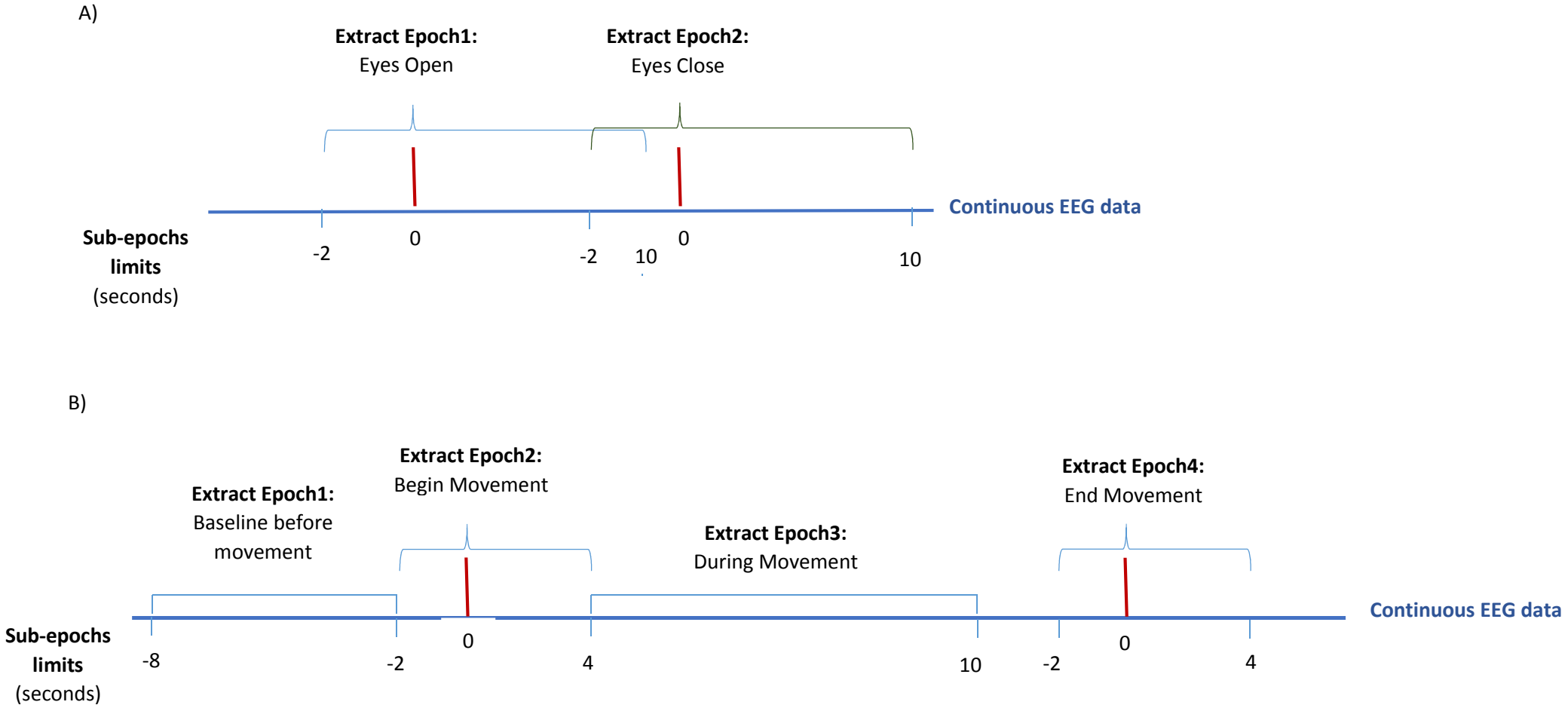


Figure 17. Sub-epochs extraction scheme. A) Represents the limits for the sub-epochs extracted for continuous EEG data for eyes open and close. B) Represents the limits for the sub-epochs extracted for continuous EEG data for right/left/both arms and hands during motor tasks.

Quantification of alpha and beta power

We used a script, for all subjects, to quantify the power of alpha between 8-10Hz, 10-12Hz and beta between 15-25Hz for movements, on the pre-cTBS and on the post-cTBS condition. The epoch limits were from -2000 to 4000ms and it was divided into six periods of one second and then, the alpha and beta frequencies for each period were quantified. The channels selected for the right upper limb were FC1, FC3, C1, C3, CP1, CP3 and CZ, for the left upper limb were FC2, FC4, C2, C4, CP2, CP4 and CZ and for both upper limbs were FC1, FC2, FC3, FC4, C1, C2, C3, C4, CP1, CP2, CP3, CP4 and CZ. These electrodes were selected independently of the group assignment. When the individual had the eyes closed the alpha was quantified between 8-13Hz and the channels selected were P7, P5, P3, P1, PZ, P2, P4, P6, PO7, PO5, PO3, POZ, PO4, PO6, PO8, O1, O2 and OZ. The time limits were between -2000 and 10000ms, and the alpha was quantified for all epoch. The quantification for the eyes closed was also performed for the pre-cTBS and for the post-cTBS condition.

- Alpha and beta power descriptive analysis

The quantification obtained by the script allowed us to construct box-and-whiskers plots, with the power of the studied frequencies in the y-axis during the period of interest (x-axis), using GraphPad Prism®. The median was used to compare the results and the whiskers represent the minimum and maximum values.

This study was performed for all the controls, dividing them into two groups, according to the stimulated hemisphere. In addition, this analysis was also performed for the patient and the matched-control, individually.

The resultant graphs were a valuable tool to visualize more clearly if the cTBS protocol caused any change in the brain's physiology, through the time, for all the experimental tasks.

- Inferential Statistics

The statistical analysis was carried out only for both control groups (right or left hemisphere stimulated) since the number of patients was not enough to perform it.

As we had five subjects stimulated on the right hemisphere and six for the left hemisphere, we chose a nonparametric test, which is more reliable for small samples. We used the Wilcoxon test, with a confidence interval of 95%, to evaluate if there were significant differences in the alpha and beta power caused by the inhibitory protocol.

Therefore, we considered that when p value was inferior to 0.05 there were significant differences.

Topographic maps and time-frequencies

After processing all the datasets, for the patient and the matched-control, we did a multistudy for each condition to generate the topographic maps for all channels, except M1 and M2. The ERD/ERS patterns induced by the two types of motor tasks was studied through the topographic maps because this method allows to inspect the spectral power changes during the recorded EEG relative to the stimulus (Yi *et al*., 2014). Topographic maps were made for each condition for alpha between 8-10Hz and 10-12Hz and beta 15-25Hz and the color limits were between -5 and 5dB. The conditions were analyzed before and after cTBS protocol. Each condition was:

- Eyes Closed;
- Right Arm Elevation movement onset;
- Left Arm Elevation movement onset;
- Both Arm Elevation movement onset;
- Right Thumb Opposition movement onset;
- Left Thumb Opposition movement onset;
- Both Thumb Opposition movement onset

The subject had his eyes closed for 10 seconds and the time was divided in five parts, so the topographic maps were calculated between 0-2000ms, 2000-4000ms, 4000-6000ms, 6000-8000ms and 8000-10000ms. The limits epoch for the movement onset was between -2000 to -4000ms, and the baseline before movement was between -8000 to -2000ms. The topographic maps were calculated in seven parts, -3000 to -2000ms, -2000 to -1000ms, -1000 to 0ms, 0 to 1000ms, 1000 to 2000ms, 2000 to 3000ms and 3000 to 4000ms.

Then we computed time-frequency plots to analyze the changes of time and frequency simultaneously, for the patient and the matched-control, when the subjects were with eyes closed and to the movement's conditions. The conditions used to perform time-frequency for right arm/hand, left arm/hand and both arm/hand were:

- Before movement onset;
- Begin Movement;

- During Movement;
- End Movement

The channels choose were C3 and CP3 for right arm/hand movements, C4 and CP4 for left arm/hand movements, C3, CZ and C4 for both arm/hand movements and the frequency limits that was selected were between 3-40Hz with padding 4. The sub epochs time limits were between -2000 to 4000ms when begin and end the movement and -8000 to -2000ms before movement and 4000 to 10000ms during movement, with 400 time points. The time-frequency when the patient had the eyes closed the channels choose were O1 and O2 and the frequency limits that was selected were between 3-40Hz with padding 4. The sub epochs time limits were between -2000 to 10000ms, with 400 time points. For the motor tasks and when the subjects had the eyes closed the wavelet cycles were 3 cycles at 0.8Hz and the color limits were between -5 and 5dB.

5. RESULTS

5.1 Patients who did not participate in the study

For sixteen weeks we went to CHUC every week, in order to check if there were patients to participate in our study. Seventeen stroke patients did not join the study due to three major reasons (which are represent in the following graph, figure 18). The main cause not to join the study was patients' stability (some were not clinically clear to do so – where 4 in 9 patients got a respiratory infection). Others simply chose not to collaborate. The remaining two main causes were due to the lack of confidence to participate in a study and demographic circumstances.

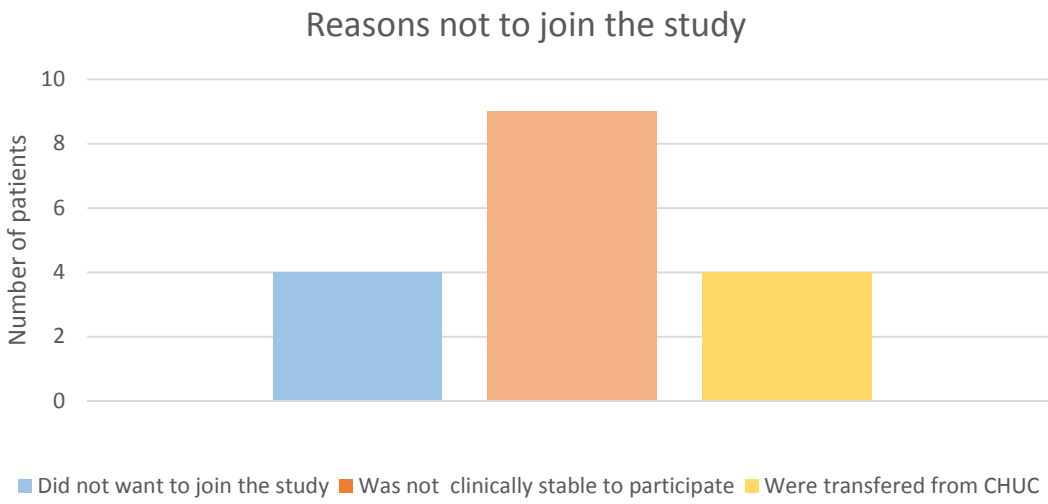


Figure 18. Reasons not to join the study

5.2 Results for the controls

Next, we are going to present the results for both control groups for each condition and the effects of cTBS over time will be analyzed comparing to the pre-cTBS condition. The conditions presented follow the subsequent order:

- Eyes closed before and after cTBS;
- Right arm elevation before and after cTBS;
- Left arm elevation before and after cTBS;
- Both arm elevation before and after cTBS;
- Right thumb opposition before and after cTBS;
- Left thumb opposition before and after cTBS;

- Both thumb opposition before and after cTBS.

The power quantification for the lower and higher alpha and for the beta band will be presented, first for the group stimulated on the right hemisphere and then, for the left hemisphere. The plots were generated with the same scale for every conditions, in order to ensure correct comparisons between graphs. However, sometimes this was a limitation because we were not able to visualize on the graphs the statistical significant differences revealed by the Wilcoxon test.

In the end of this section we present a summary (table 5) with the global tendency for the variation on alpha and beta power quantification after the cTBS protocol, over time.

- **Eyes Closed**
    - **Controls stimulated in the right hemisphere**

Through the observation of the graph, figure 19, we saw a difference between post and pre-cTBS on the power quantification. In fact, cTBS increased the alpha power significantly ($p < 0.0001$).

- **Controls stimulated in the left hemisphere**

For controls stimulated in the left hemisphere we saw a decrease of the alpha power after the cTBS protocol, figure 20. This power decrease was statistical significant ($p< 0.0001$).

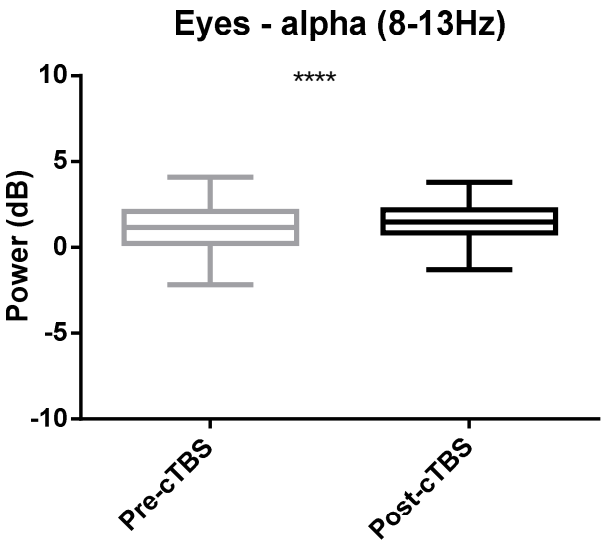


Figure 19. Quantification graphs for controls stimulated in the right hemisphere with eyes closed.

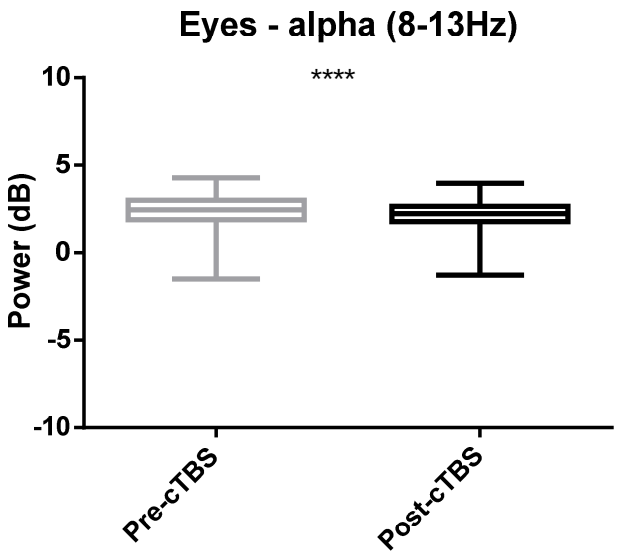


Figure 20. Quantification graphs for controls stimulated in the left hemisphere with eyes closed.

- **Right Arm Elevation**

    - **Controls stimulated in the right hemisphere**

When subjects elevated the right arm, we observed a difference for the lower and higher alpha between pre and post-cTBS conditions. After cTBS, there was a statistically significant increase of the alpha power between 8-10Hz and 10-12Hz. This increment was more pronounced from -1000ms ($p < 0.0001$). For the beta band we could also see a power increase after the cTBS. There was a statistically significant increase from -1000ms ($p<0.0001$). The obtained graph was presented as supplementary material on CD (figure A6).

    - **Controls stimulated in the left hemisphere**

This motor task showed for this group of subjects an increase of the lower alpha power after the cTBS condition and there was a statistically significant increase for all periods ($p<0.0001$), except between 2000 and 3000ms, where was not seen any differences ($p=0.8398$). For the higher alpha was also seen an increase of power after the cTBS and this was more statistical significant between -1000 and 2000ms ($p<0.0001$). However, we did not see any statistical differences between 3000 and 4000ms ($p=0.2394$). In the beta band along time and among both conditions (pre and post-cTBS) we saw a negative power. For the post-cTBS condition we could see a decrease of beta power in the last three seconds and this was statistical significant (1000 to 2000ms: $p=0.0064$; 2000 to 4000ms: $p<0.0001$). In the first second and third second we did not see any statistical differences ($p=0.2571$, $p=0.1058$, respectively). The obtained graph was presented as supplementary material on CD (figure A7).

- **Left Arm Elevation**

    - **Controls stimulated in the right hemisphere**

For the lower alpha we have an increase of power after the cTBS and this difference was statistical significant for almost all periods (-2000 to -1000ms: $p=0.0129$; -1000 to 2000ms: $p<0.0001$). Therefore, the difference between pre and post-cTBS was not significant only between 2000 and 3000ms ($p=0.5302$). For the higher alpha we also have an increase of power on the post-cTBS condition and this was more significant between -1000 and 3000ms ($p<0.001$). In the first and last second the difference was less significant ($p=0.0012$ and $p=0.0176$, respectively). After the cTBS there was a

significantly increase of the beta power over time ($p<0.0001$). The obtained graph was presented as supplementary material on CD (figure A8).

- **Controls stimulated in the left hemisphere**

When the subject performs the motor task with the left arm, the power of the lower alpha was bigger in the post-cTBS condition for almost all periods (-2000 to -1000ms: $p=0.0008$; -1000 to 0ms: $p<0.0001$; 1000 to 2000ms: $p<0.0001$; 3000 to 4000ms: $p<0.0001$). Between these periods, we did not see statistical differences. For the higher alpha and beta band was seen statistical significant differences between before and after cTBS over time. For the higher alpha and beta band, in the first second, the increase of power after the cTBS was not so marked ($p=0.0023$; $p=0.0054$, respectively) and from -1000 to 4000ms we have the strongest statistical difference between both conditions ($p<0.0001$). The obtained graph was presented as supplementary material on CD (figure A9).

- **Both Arm Elevation**

  - **Controls stimulated in the right hemisphere**

For the both arms elevation, the lower alpha showed a power increase on the post-cTBS condition and this was statistical significant between 1000 and 3000ms ($p<0.0001$). In the first and last second the difference was not so significant ($p=0.0218$ and $p=0.0015$, respectively) and we saw a power decrease after TMS. Between -1000 and 1000ms there were not statistical differences between both pre and post-cTBS conditions. Analyzing the higher alpha we could observe differences between both conditions in two different periods (-2000 to -1000ms and 1000 to 3000ms: $p<0.0001$), where we could see in the first period a power decrease after TMS, and then, a power increase, respectively. The difference between pre and post-cTBS was not significant between -1000 and 1000ms and in the last second. The beta power in the post-cTBS condition showed a significantly increase of power over time ($p<0.0001$). The obtained graph was presented as supplementary material on CD (figure A10).

  - **Controls stimulated in the left hemisphere**

Analyzing the lower alpha, the graph showed statistical differences between both pre and post-cTBS conditions in almost all periods (-2000 to -1000: $p<0.0001$; -1000 to 0ms: $p=0.0019$; 1000 to 2000ms: $p=0.0002$; 3000 to 4000ms: $p<0.0001$). In the first period the alpha power decreases, then in the second and third period the power increases

and in the last period, the power decreases. Overall, the power of the lower alpha decreases after the TMS. So, in the third and last second was not seen statistical differences between pre and post-cTBS condition (p=0.6049 and p=0.4903, respectively). For the higher alpha and beta band we have a decrease of power after the cTBS and this difference was statistical significant for all periods (p<0.0001). The obtained graph was presented as supplementary material on CD (figure A11).

- **Right Hand Opposition**

    - **Controls stimulated in the right hemisphere**

For this motor task the lower and the higher alpha have a decrease of power on the post-cTBS comparing to the pre-cTBS condition. For the lower alpha the statistical significant differences were seen for three periods (-1000 to 0ms and 3000 to 4000ms: p<0.0001) and in the first second is less significant (p=0.0003). The period (1000 to 2000ms: p<0.0001) is the only period that we can see an alpha increase after the TMS. From 0 to 1000ms and 2000 to 3000ms differences were not significant. For the higher alpha the difference between before and after cTBS were statistical significant throughout most of the period (p<0.0001), except between 1000 and 2000ms where there were not seen significant differences (p=0.0624). For the beta band was seen an increase of power after the cTBS and this difference was statistical significant for almost all periods (-2000 to -1000ms: p<0.0001; 1000 to 2000ms: p<0.0001 and 2000 to 3000ms: p=0.0004). For one second (-1000 to 0ms: p=0.0013) was seen a power decrease. Therefore, the difference between pre and post-cTBS was not significant between 0 and 1000ms (p=0.7909) and in the last second (p=0.0858). The obtained graph was presented as supplementary material on CD (figure A12).

    - **Controls stimulated in the left hemisphere**

For the lower and higher alpha we have an increase of power after the cTBS comparing to pre-cTBS condition and this difference was statistical significant for all periods (p<0.0001). For the beta band we also see an increase of power on the post-cTBS condition and the differences between both conditions were statistical significant for almost periods (-1000 to 0ms: p<0.0001; 0 to 1000ms: p=0.0012; 1000 to 2000ms: p=0.0009; 2000 to 3000ms: p<0.0001; 3000 to 4000ms: p=0.0381). So, in the first second the differences were not statistical significant (p=0.5025). The obtained graph was presented as supplementary material on CD (figure A13).

- **Left Hand Opposition**

    - **Controls stimulated in the right hemisphere**

For the left hand opposition in the lower and higher alpha we have an increase of power after the cTBS in comparison to the pre-cTBS condition and this difference was statistical significant for all periods ($p<0.0001$). Overall, for the beta band the power was increased after the cTBS and it was statistical significant for almost all periods (-1000 to 0ms: $p=0.0008$; 0 to 1000ms: $p=0.0025$). In certain periods (-2000 to -1000ms, 1000 to 2000ms and 3000 to 4000ms: $p<0.0001$) instead of seeing an increase, we observe a power decrease. The only period that was not seen statistical differences between conditions was from 2000 to 3000ms. The obtained graph was presented as supplementary material on CD (figure A14).

    - **Controls stimulated in the left hemisphere**

The lower alpha had an increase of power after the cTBS and this was statistical significant for all periods (-2000 to -1000: $p=0.0020$; -1000 to 3000: $p<0.0001$; 3000 to 4000ms: $p=0.0015$). For the higher alpha was also seen a power increase and it was statistical significant for all periods (-2000 to -1000: $p=0.0033$; -1000 to 3000: $p<0.0001$; 3000 to 4000ms: $p=0.0212$). For the beta band was seen a significant power decrease after cTBS from -2000 to 1000ms and 2000 to 3000ms ($p<0.0001$). The obtained graph was presented as supplementary material on CD (figure A15).

- **Both Hand Opposition**

    - **Controls stimulated in the right hemisphere**

The lower and higher alpha had a power decrease after the TMS comparing to the pre-TMS. For the lower alpha is seen a statistical significant difference for almost all periods (-2000 to -1000ms: $p<0.0001$; 0 to 1000ms: $p=0.0018$; 2000 to 3000ms: $p=0.0098$ and 3000 to 4000ms: $p=0.0313$). Therefore, in comparison to the pre-cTBS condition, the power after cTBS did not show statistical differences in two periods, -1000 to 0ms and between 1000 and 2000ms. For the higher alpha was also seen statistical differences after the cTBS for almost all periods (-2000 to -1000ms: $p=0.0030$; 0 to 1000ms: $p=0.0006$; 1000 to 2000ms and 2000 to 3000ms: $p<0.0001$). The higher alpha did not show statistical differences between -1000 and 0ms and in the last second. Analyzing the beta band it was observed a power increase and this was statistical different between -1000 and 4000ms

($p<0.0001$), except the interval between 1000 and 2000ms ($p=0.0273$). The only period that was not seen statistical differences it was in the first second. The obtained graph was presented as supplementary material on CD (figure A16).

- **Controls stimulated in the left hemisphere**

We could observe a statistical significant power increase for the lower alpha after cTBS only for certain periods of time (-1000 to 0ms and 2000 to 3000ms: $p<0.0001$; 0 to 1000ms: $p=0.0037$). In the other periods, there were not seen statistical differences. The higher alpha had a significant power increase after the cTBS protocol in the first three seconds and also in the fifth second (-2000 to 0ms and 2000 to 3000ms:$p<0.0001$; 0 to 1000ms: $p=0.0001$).The beta band between pre and post-cTBS conditions showed statistical significant power decrease for almost periods from 0ms ($p<0.0001$). The only period that was not seen statistical differences was in the first second ($p=0.3000$). The obtained graph was presented as supplementary material on CD (figure A17).

Below, it is presented the summary table (table 5) to clarify the global tendency of the described alterations after the protocol. When the lower and higher alpha showed the same tendency, we designated both as alpha.

Summary Table Quantification Graphs		
Tasks	**cTBS protocol is applied on the control group on the right hemisphere**	**cTBS protocol is applied on the control group on the left hemisphere**
Eyes close	Alpha ↑	Alpha ↓
Right Arm	Alpha ↑ Beta ↑	Alpha ↑ Beta ↓
Left Arm	Alpha ↑ Beta ↑	Alpha ↑ Beta ↑
Both Arm	Alpha ↑ Beta ↑	Alpha ↓ Beta ↓
Right Hand	Alpha ↓ Beta ↑	Alpha ↑ Beta ↑
Left Hand	Alpha ↑ Beta ↑	Alpha ↑ Beta ↓
Both Hand	Alpha ↓ Beta ↑	Alpha ↑ Beta ↓

Table 5. Summary table for the quantification graphs of alpha and beta power for each control group after cTBS protocol.

5.3 Results for the matched-control and stroke patient

Next, we will present the results obtained for the matched-control followed by the stroke patient to compare the results. The conditions presented follow the same order that was chosen for the controls. Movements performed with the left upper-limb were imagined by the patient because he was plegic and, therefore, was not able to move the left arm/hand. Both subjects were stimulated on the left hemisphere, since the patient had the stroke on the right hemisphere.

It will be presented for each condition (pre and post-cTBS) the results obtained for the topographic maps, then for the time-frequency, and finally, the power quantification for the lower and higher alpha and for the beta band. These three analysis have the main goal to compare and characterize the effects on the alpha and beta band, induced by the cTBS protocol. For the topographic maps and time-frequency we also evaluate the main changes for each condition over time, analyzing also the baseline period before and after cTBS.

The topographic maps and the time-frequency represent the power for the frequencies in study. If we obtain a blue topography, it means that the power is negative, and therefore, the brain is more activated. This activation correlates with the ERD, which was already described. The ERS is seen when the brain's topography is red/yellow and this means the brain is deactivated. The scale bar is imperative to verify when the power level for each frequency is more positive or negative.

The global changes on brain's topography after the protocol are summarized on table 6, in the end of this section. As we did for the group analysis, it is also presented a summary (table 7) with the global tendency for the variation on alpha and beta power quantification after the cTBS protocol, over time.

- **Eyes closed between 8-13Hz**
    - **Matched-control**

When the matched-control was with the eyes closed, the topographic maps for frequencies between 8-13Hz, showed an evident different pattern before and after cTBS was applied on the left hemisphere. After the protocol, the brain's topography was more negative and the right hemisphere was more activated, figure 21. Knowing that cTBS protocol, when applied in the left hemisphere, it becomes more deactivated. Therefore,

the right hemisphere became more activated, the results are according what has been described in the literature.

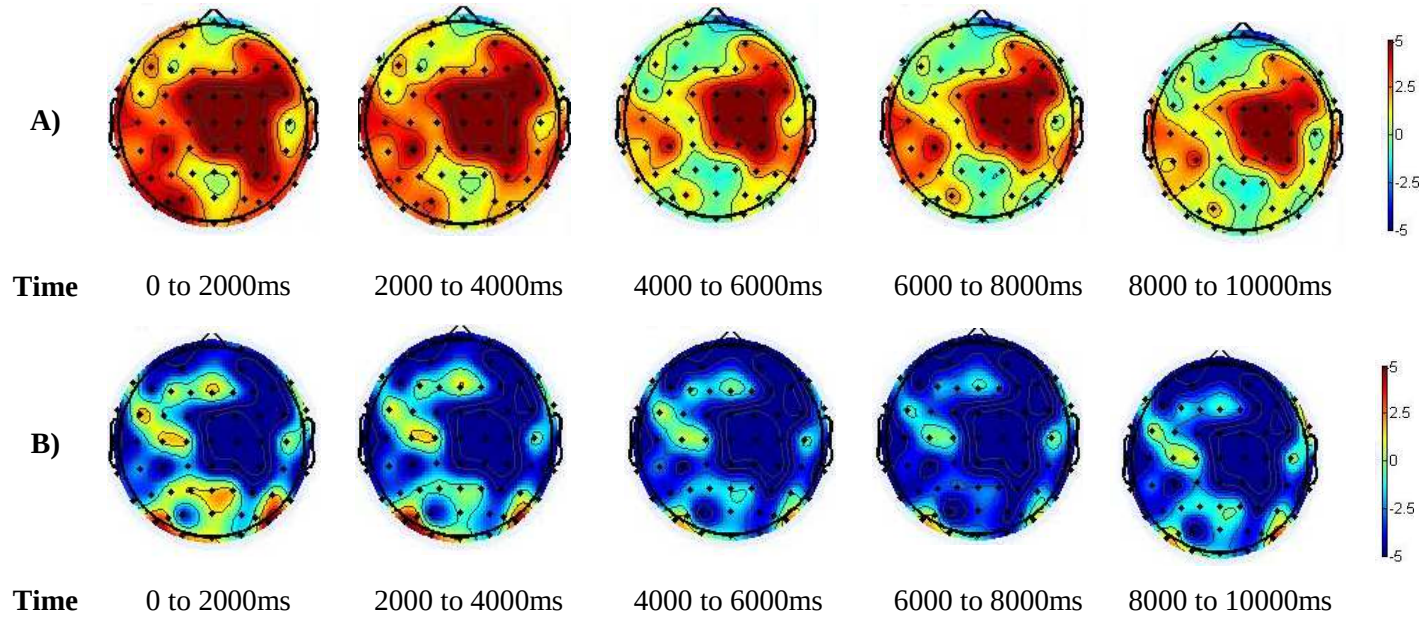


Figure 21. Topographic maps for matched-control - The topographical distribution within alpha band for ten seconds divided in five periods of 2000ms. A) Represents before cTBS stimulation. B) Represents after cTBS stimulation on the left hemisphere.

- **Stroke Patient**

In the topographic maps it was clear a focus on the right hemisphere, which became more evident after the cTBS. The focus appeared to decrease the alpha power after cTBS protocol. As we saw for the matched-control, after cTBS protocol, the brain's topography was also more negative, figure 22.

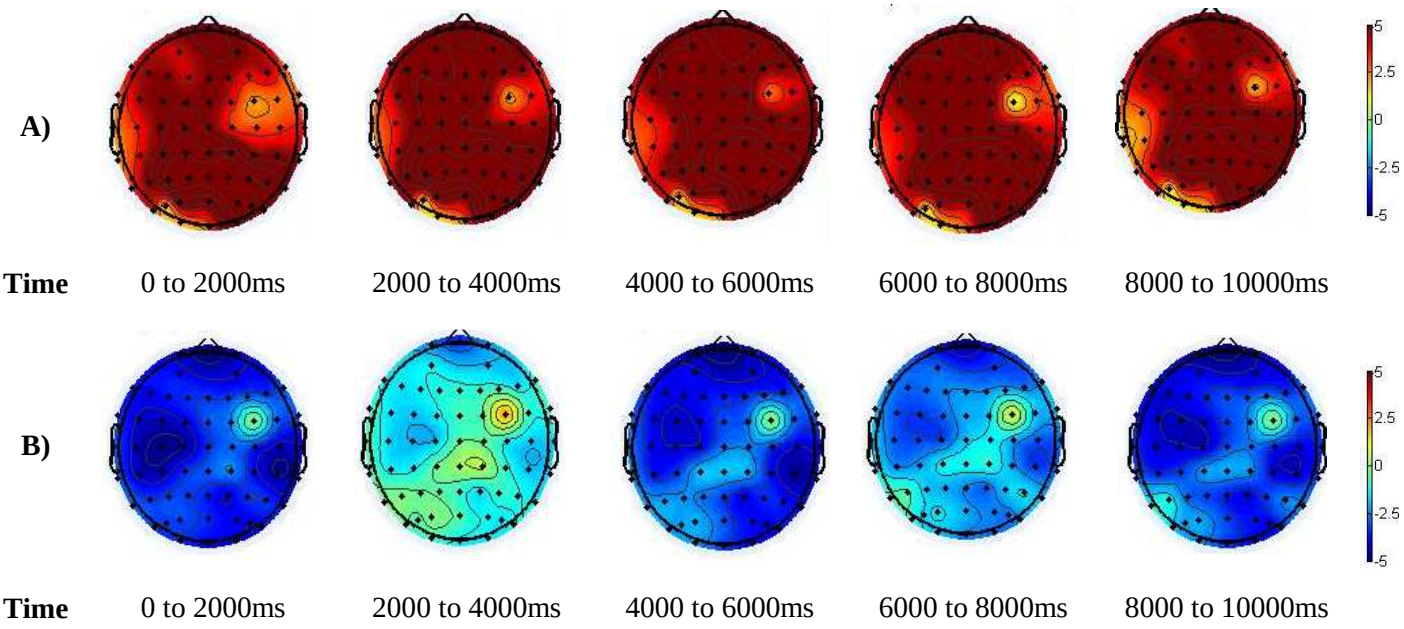


Figure 22. Topographic maps for stroke patient - The topographical distribution within alpha band for ten seconds divided in five periods of 2000ms. A) Represents before cTBS stimulation. B) Represents after cTBS stimulation on the left hemisphere.

- **Time Frequency: Eyes Closed**
  - **Matched-control**

The time-frequency for the electrode O1 showed an increase of alpha after cTBS, due to an increase of inhibition on the left hemisphere. On the topographic maps, figure 21, we have described a decrease of alpha on brain's topography after the protocol was applied on the left hemisphere; nevertheless the positivity for the alpha band was also seen on the posterior regions of the brain. Even though, for the electrode O2 there were not verified significant alterations, between before and after cTBS protocol, figure 23.

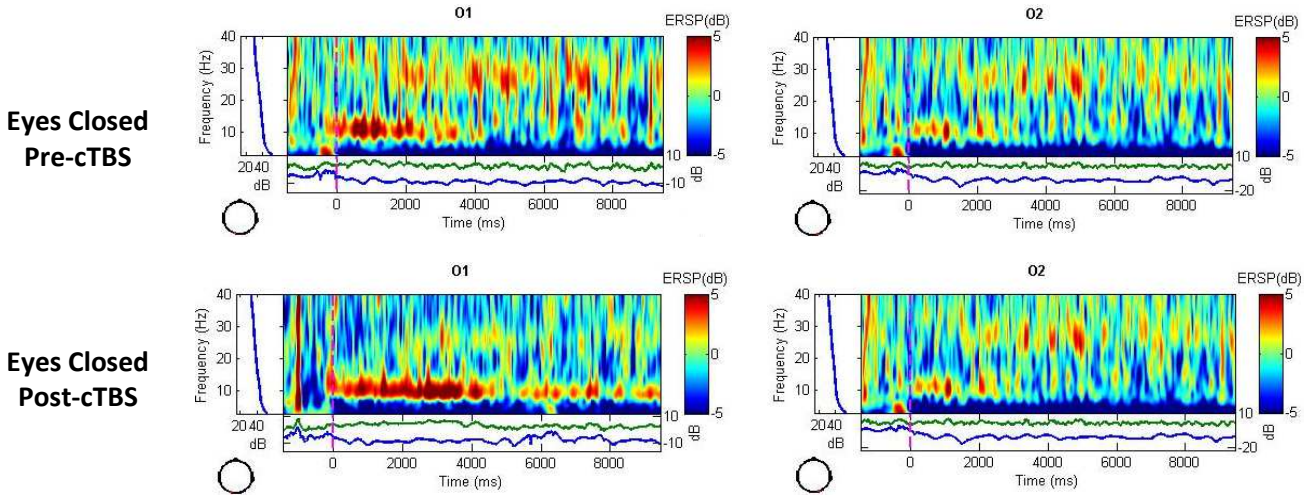


Figure 23. Time-frequency for matched-control - channels 01 and 02 between 3-40Hz in two different conditions for eyes closed: before and after cTBS on the left hemisphere.

- **Stroke patient**

The patient had the stroke in the right hemisphere and we could observe in the time-frequency figure, before cTBS protocol, an alpha pattern for the electrode O2. The same pattern was not detected for the electrode O1, because the activity of the left hemisphere was pathologically increased. After the inhibitory protocol, we could see an evident reduction of alpha in the electrode O2, which was the excited hemisphere (figure 24). There were no significant changes between the electrode O1 and O2, which could be due the interhemispheric connections and a rebalance activity between both hemispheres, induced by the TMS.

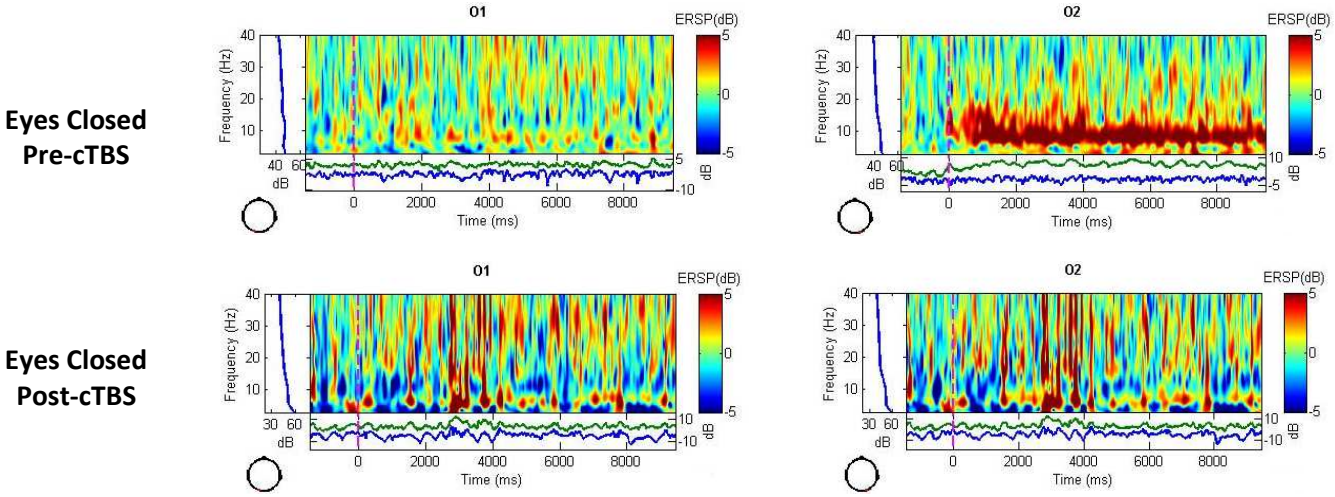


Figure 24. Time-frequency for stroke patient - channels 01 and 02 between 3-40Hz in two different conditions for eyes closed: before and after cTBS on the left hemisphere.

- **Quantification Graphs: Eyes Closed**
  - **Matched-control**

Between both pre and post-cTBS conditions the alpha had a negative power. When the subject had his eyes closed before the cTBS the brain had more alpha compared to the post-cTBS condition, figure 25. So, the brain was more deactivated. After the cTBS, we had less alpha, therefore, the brain was more activated.

  - **Stroke patient**

When the patient had his eyes closed, the alpha had a positive power in the pre-cTBS condition. In the post-cTBS condition there was a marked decrease in the amount of power, so, the brain was also more activated, figure 26.

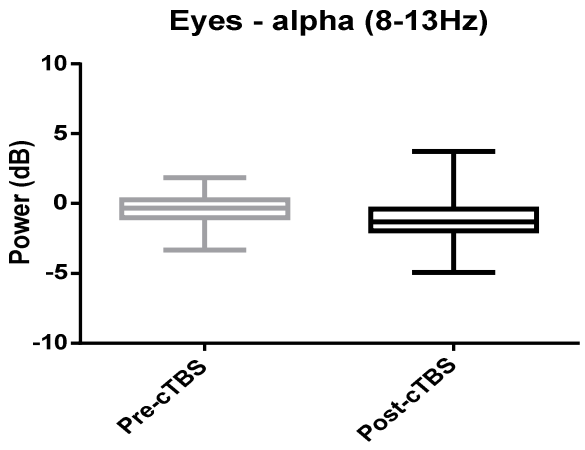


Figure 25. Quantification graphs for matched-control with eyes closed before and after cTBS on the left hemisphere.

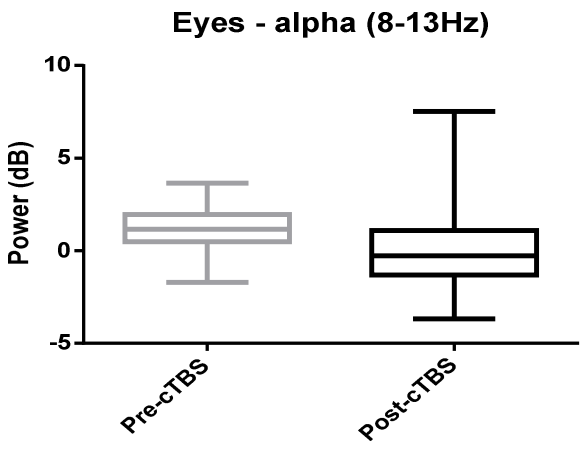


Figure 26. Quantification graphs for stroke patient with eyes closed before and after cTBS on the left hemisphere.

- **Right Arm Elevation between 8-10Hz**

  - **Matched-control**

Before the cTBS protocol, two seconds before the subject elevated the right arm it was observed in the midline brain over the central and parietal electrodes sites an alpha focus. This focus was detected in the midline between -2000 and 1000ms. After this period, the focus started to become noticeable on the right hemisphere and became more deactivated over time. As the focus became more positive, the surrounding areas became more activated. After the TMS, the same focus was seen in a topography more posterior as we saw before the TMS and it was more activated. We could see it from -2000 to -1000ms, and over time we could visualize it with a topography extending to the frontal, parietal and central areas, but was always more negative than the rest of the brain. In the last three seconds, we started to see a deactivated focus over the frontal areas. The obtained topographic maps were presented as supplementary material on CD (figure A18).

  - **Stroke Patient**

Three seconds before the stroke patient raises the arm, we could see a negativity on the left hemisphere that begins to disappear and on the right hemisphere we also see a negativity in the last second before the movement initiation. This focus was observed on the right hemisphere and, in the following two seconds, spreads to the left hemisphere. Once the subject elevated the arm, we could see a focus of alpha on the midline over fronto-central areas that over time, will be spreading and was becoming more deactivated.

In the last second the right hemisphere, which was the affected hemisphere, had a strong alpha power that was a match with the inhibition induced by the lesion. In the post-cTBS condition, the brain was more activated. One second before the movement begins, the right hemisphere was more negative over central, parietal and temporal areas, and in the following second, the negativity spreads all over the brain, but was stronger on the right hemisphere. This strong negativity begins to fade over time, but the right hemisphere was still more negative than the left, because the cTBS protocol induced an excitation on the contralateral hemisphere as it was hypothesized. The obtained topographic maps were presented as supplementary material on CD (figure A19).

- **Right Arm Elevation between 10-12Hz**
    - **Matched-control**

The higher alpha was almost similar to the lower alpha in both conditions, pre- and post-cTBS. However, after the TMS the higher alpha, in the period -2000 to -1000ms, the focus was smaller and the negative power along time seems to be minor, compared to the post-cTBS condition for the alpha between 8-10Hz. The obtained topographic maps were presented as supplementary material on CD (figure A20).

- **Stroke Patient**

The alpha between 10-12Hz had the same topography and power compared to the lower alpha, before and after cTBS. The obtained topographic maps were presented as supplementary material on CD (figure A21).

- **Right Arm Elevation between 15-25Hz**
    - **Matched-control**

For the beta band we could consider the focus on the fronto-central areas could represent the movement. Two seconds before the movement, the focus was more deactivated compared to the next second. There were a reduction of alpha power in this period. Along time, this focus maintains the power and the surrounding areas from the period -1000 to 1000ms became more activated and, in the following seconds, were more positive. The topography was more focused on the midline over fronto-central electrodes sites, except in the last second of movement. In the post-cTBS condition, two seconds before the movement, the focus analyzed previously, was now more activated. In the following seconds, the negativity spreads all over the brain, but a more activated focus is

detectable compared to the rest of the brain, over fronto-central electrodes sites. When the subject elevated the right arm and maintain the arm in the air, this focus becomes smaller over time. The obtained topographic maps were presented as supplementary material on CD (figure A22).

- **Stroke Patient**

For the beta band, two seconds before the movement, in the frontal-central electrodes sites, we can see a deactivation, which became less positive in the following two seconds. In the period from 1000 to 4000ms this focus becomes once again more positive and bigger. The surrounding areas were more negative, but this negativity was seen best from -1000 to 1000ms, and then started to disappear. In the post-cTBS condition, the anterior focus was more activated and the areas surrounding were now more positive. This activation achieves was maximum in the first second after the patient raises the arm. The obtained topographic maps were presented as supplementary material on CD (figure A23).

- **Time Frequency: Right Arm Elevation**

  - **Matched-control**

This motor task did not show a well-defined activation when the movement begins. Mainly over the electrode C3, we could see -500ms before the movement begins an activation, but when the subject elevated the arm, this activation begins to disappear gradually. When the subject lowers the arm, we could see a negativity especially for the high frequencies (±30Hz), which is followed by a deactivation of frequencies between ±12-22Hz. We assume that the negativity seen after the movement stops can be correlated to the movement of lowering the arm. After the TMS, we could see better an activation when the subject elevated the arm, but when the movement stopped, the deactivation seen previously was not so strong. The obtained time-frequency was presented as supplementary material on CD (figure A24 and A25).

  - **Stroke Patient**

For the stroke patient we cannot see a precise activation when the subject raises the arm and a deactivation when ends the movement. However, when the patient stopped the movement, over the electrode C3 there was a positivity, which could derive from a deactivation. After the TMS, we could see a clear pattern of activation when the subject

raises the right arm. When move downwards the right arm, we could see an activation, from 1000 to 1500ms, over C3 and CP3 electrodes, followed by a subtle deactivation. It seems to have a deactivation for the higher frequencies, when the subject was at rest. The obtained time-frequency was presented as supplementary material on CD (figure A26 and A27).

- **Quantification Graphs: Right Arm Elevation**

    - **Matched-control**

The alpha power between 8-10Hz was bigger after the TMS protocol. For the higher alpha we also see an increased power after the cTBS, excepted between 1000 and 3000ms. Overall, after the TMS, we saw a superior increase for the lower alpha than for the higher alpha. The alpha quantification before and after TMS did not reveal important variations over time. For the beta band, there was also an increase of the power after the protocol, except from 1000 to 3000ms, as it has been observed for the higher alpha. The obtained graph was presented as supplementary material on CD (figure A28).

    - **Stroke Patient**

The lower alpha in the first second had more power after the TMS. In the following seconds the power decreases. The higher alpha in the post-cTBS condition, was also bigger in the first second, and then, there was a reduction of its power. So, overall, before the TMS we have more power of alpha between 8-10Hz and 10-12Hz. For the beta band, we also have less beta power after the TMS from -1000ms. This decrease of power was more evident after 1000ms. The obtained graph was presented as supplementary material on CD (figure A29).

- **Left Arm Elevation between 8-10Hz**

    - **Matched-control**

Two seconds before the subject raised the left arm, we could see a focus over the both hemispheres, but was more pronounced on the right hemisphere over the frontal and central electrode sites. This focus became more negative in the next second. In the following seconds, this focus became more deactivated over time. The surrounding areas around were more negative during the motor task compared to the focus. After the TMS,

this focus was seen more negative and more posteriorly. This focus was seen over the centro-parietal electrodes sites on the right hemisphere and in the lasts seconds spreads to the left hemisphere. Over all, the brain was more activated comparing to the pre-cTBS condition. The obtained topographic maps were presented as supplementary material on CD (figure A30).

- **Stroke Patient**

When the stroke patient imagines to raise the left arm in the pre-cTB condition the brain was more deactivated. As time moved forward, we could observe a clear focus on both hemispheres over the centro-parietal electrodes sites. These activation focus begins before the stroke patient started to imagine the movement and then increases with the beginning of the movement, and remains constant, except from the period 1000 until the 2000ms. After the TMS, three seconds before the motor task the brain was more positive. The activated focus seen on the pre-cTBS condition, was replaced by a deactivated focus, on the post-cTBS condition, that became lateralized to the left hemisphere over the centro-parietal electrodes sites. This deactivation focus reached its maximum from 2000 to 3000ms, while the rest of the brain was activated. The obtained topographic maps were presented as supplementary material on CD (figure A31).

- **Left Arm Elevation between 10-12Hz**

  - **Matched-control**

The differences between the lower and the higher alpha were not detected. The obtained topographic maps were presented as supplementary material on CD (figure A32).

  - **Stroke Patient**

It was not seen a significant difference between the lower and the higher alpha, except for a stronger activation for the lower alpha post-cTBS, comparing to the higher alpha. The obtained topographic maps were presented as supplementary material on CD (figure A33).

- **Left Arm Elevation between 15-25Hz**

    - **Matched-control**

From the period -2000 to -10000ms the positivity seen on the both hemispheres became more negative in the next second. Although, in the following seconds the negativity was more pronounced on the left hemisphere than on the right hemisphere. From the period -1000 to 0ms we could see on the right hemisphere over the fronto-central electrodes sites a focus, which became more negative than the previous second, but more positive comparing the surround areas. This focus became slightly deactivated over time. In the post-cTBS condition, from -1000 to 2000ms, the brain was significantly more activated comparing to the condition pre-cTBS. We could see on the right hemisphere a clear activation of the beta band over the fronto-central electrodes sites. This negativity, increases from -1000 to 1000ms, and then becomes more positivity over time. The obtained topographic maps were presented as supplementary material on CD (figure A34).

    - **Stroke Patient**

For the beta band the pre-cTBS condition was also more negative than the post-cTBS condition. This activation spreads from the right hemisphere to the left hemisphere, and we were able to see it from -2000 to 0ms. Afterwards, this activation begins to increase over the centro-parietal electrodes sites over the right hemisphere, and on the left hemisphere was more pronounced on the central electrodes sites. In the next two seconds, this negativity spreads to all brain and in the last second, the activation decreases. After the TMS, the brain was clearly more positive before the patient started to image the movement. One second before the motor tasks begins, we could see a negativity appearing over the fronto-central sites over the right hemisphere. This negativity became more negative when the patient started to imagine the movement and spreads to the left hemisphere. The activation was more pronounced on the fronto-central electrodes sites on both hemispheres. The obtained topographic maps were presented as supplementary material on CD (figure A35).

- **Time Frequency: Left Arm Elevation**

  - **Matched-control**

The time-frequency for the electrodes C4 and CP4 showed an activation for the frequencies between ±10-30Hz from the -500ms when the subject elevated the left arm. Then, we could see a deactivation for lower and higher frequencies and an activation between ±20-35Hz. When the subject lowered the arm, there was an activation during these period, and then we could see a deactivation on both electrodes. After the TMS, we could observed well-defined negativity when the subject raises the arm, mainly over the electrode C4. When the subject stopped to perform the motor task, we did not see a deactivation, as we supposed to see. The obtained time-frequency was presented as supplementary material on CD (figure A36 and A37).

  - **Stroke Patient**

When the patient begins to imagine the movement an activation occurs for the lower frequencies and a deactivation for the higher frequencies, and this pattern was more evident on the electrode C4. Ending the movement, we see an activation for lower and higher frequencies, approximately, from 400ms. After the 1250ms the negativity was more marked for the higher frequencies over the electrode C4. After the TMS, when the patient imagines to raise the left arm, we could see an activation in the first 500ms for the higher and lower frequencies over the electrode C4, and then we only verify for the lower frequencies. For the electrode CP4 we only see for an activation for the lower frequencies. A clear deactivation was seen for the higher and medium frequencies for both channels. When the subjects stopped to imagine the movement, in the first 1000ms on the electrode C4, we see a negativity for lower and higher frequencies, and then we begin to see a positivity for lower and medium frequencies. The obtained time-frequency was presented as supplementary material on CD (figure A38 and A39).

- **Quantification Graphs: Left Arm Elevation**

  - **Matched-control**

Overall, the graph showed that the post-cTBS condition increases the power of the lower and higher alpha compared to the pre-cTBS condition, except from the period

between 0 and 2000ms. The beta band had a greater power after the TMS. The obtained graph was presented as supplementary material on CD (figure A40).

- **Stroke Patient**

For the lower and higher alpha we have more power before the cTBS condition between -2000 and -1000ms, 1000 and 2000ms and between 3000 and 4000ms. In the other periods, after the TMS, we have more power. For the beta band, we have a bigger power in the pre-cTBS condition, except from -2000 to -1000ms. The obtained graph was presented as supplementary material on CD (figure A41).

- **Both Arm Elevation between 8-10Hz**

  - **Matched-control**

For the both arms elevation we could see a negativity on the frontal, parietal and occipital electrodes sites on both hemispheres from -2000 to -1000ms and then we see again from 1000 to 3000ms. Between these periods in the specific areas the negativity decreases. On midline over the fronto-central electrodes sites, from -2000 to 1000ms, the alpha had a positive power, and in the next two seconds, it became less deactivated. This deactivation was more defined and more spread during the first second after the motor task onset. After this period, the deactivation of the alpha power, decreases in the next second, and then, increases again and starts to lateralize to the left hemisphere. After the TMS, the brain was more activated. Two seconds before the subjects raises the two arms we could see a negativity the fronto-central electrodes on the right hemisphere. This focus was seen in the next second over the midline, and then this negativity spreads in the brain. This activation seen achieves its maximum on both hemispheres, from 0 to 1000ms, and in the following seconds we could see a decrease of the negativity becoming more centered on the midline. The obtained topographic maps were presented as supplementary material on CD (figure A42).

  - **Stroke Patient**

For this motor task, the patient elevated the right arm and at the same time, imagines he was raising the left arm. The negativity pattern in the pre-cTBS condition was well-defined over time. This activation was seen 3000ms before the movement onset and increases, reaching a maximum 1000ms after. There were two focus 2000ms before the movement, on the right hemisphere was seen over the central electrodes and on the

left hemisphere in seen over the centro-parietal electrodes sites. In the next second, we only see the focus on the right hemisphere and in these both periods, 1000 to 2000ms and 3000 to 4000ms, the negativity on the right hemisphere was stronger than on the left one. After the TMS, the brain's topography was more positive. Two seconds before the movement onset, we can see a focus on the right hemisphere. This was more deactivated compared to the period between 0 and 1000ms. Then, in the following seconds, the focus became more positive and spread to the left hemisphere over the central, temporal and parietal areas. The surrounding areas were more activated. The obtained topographic maps were presented as supplementary material on CD (figure A43).

- **Both Arm Elevation between 10-12Hz**
    - **Matched-control**

On the pre-cTBS condition the higher alpha showed a similar topography. The negativity power seen for the higher alpha was stronger, and was seen more over the posterior areas on the right hemisphere. The alpha deactivation was also seen but it was not so positivity. In the post-cTBS condition the topography of the alpha was the same as we see for the lower alpha, but it had less negativity. The obtained topographic maps were presented as supplementary material on CD (figure A44).

- **Stroke Patient**

The pre-cTBS and post-cTBS conditions were similar as it was described for the lower alpha. The obtained topographic maps were presented as supplementary material on CD (figure A45).

- **Both Arm Elevation between 15-25Hz**
    - **Matched-control**

In the first second the brain was deactivated and in the next second became more positive. In the midline we could see a focus where the beta was more positive than the rest of the brain. In the following two seconds, this focus was less positive and the surrounding areas become more negative. After the first second of the movement onset, the negativity started to decrease and the focus in the midline became more positive and was extending to the frontal areas. On the post-cTBS condition, the focus seen previously in the pre-cTBS condition, had the same topography, but now it had a negative power. The surround areas were now more positive. In the last second, the midline focus became

more deactivated and the surrounding areas increased their positivity. The obtained topographic maps were presented as supplementary material on CD (figure A46).

- **Stroke Patient**

For the beta band, before the TMS, the brain was also more negative when compared to the post-cTBS condition. However, this negativity was not so strong, as we saw for the lower and higher alpha. The brain's topography was negative, but this was stronger after the period -1000 to 0ms and could be seen on both hemispheres, mainly over centro-parietal electrodes sites. The post-cTBS condition showed a positivity, which was more intense in the last the seconds. Two seconds before the movement onset, we see a positivity on the right hemisphere, which became less positive in the following second. From 0ms we start to observe a deactivation on the left hemisphere, spreading to the right hemisphere. Then, we see a strong deactivation extended on both hemispheres. The obtained topographic maps were presented as supplementary material on CD (figure A47).

- **Time Frequency: Both Arm Elevation**

- **Matched-control**

The time-frequency for the C3 and CZ channels reveals an activation until the first second after the movement onset. After ending the movement, there was an activation, when the subject lowers both arms, and then we saw a deactivation more pronounced on frequencies above 20Hz, on C3, CZ and C4 channels. After the TMS, when the subject lifts both arms, we see again a negativity for the electrodes C3 and CZ, and appears to be stringer when compared with the pre-cTBS condition. As soon as the movement stopped, we could see an activation appearing 1500ms on the electrode CZ, and for the electrodes C3 and C4. The obtained time-frequency was presented as supplementary material on CD (figure 48 and A49).

- **Stroke Patient**

In the pre-cTBS condition we could see a clear activation on the electrodes C3, CZ and C4, when the patient raises the right arm and imagines to elevate the left arm. This activation was better observed in the electrode C4. When the movement stopped, we did not see a positivity, as it as supposed. For the electrode C4, we see a negativity for higher frequencies. After the TMS, 500ms before and 500ms after the motor tasks begins,

we see a activation on the electrodes C3, CZ and C4, and the strongest deactivation was seen for the higher frequencies over the electrode C3. When the movement stopped, we see only a strong negativity over the electrode C3. Over the electrode C4, we see an activation for the lower frequencies when the patient ends the motor task, and a slightly deactivation for frequencies between 25-30Hz. The obtained time-frequency was presented as supplementary material on CD (figure A50 and A51).

- **Graphs Quantification: Both Arm Elevation**

    - **Matched-control**

The graph for the lower alpha reveals a reduction in power after the inhibitory protocol for all the periods, excluding from -1000 to 0ms and from 3000 to 4000ms in which we could observe increased post-cTBS. For the alpha between 10-12Hz, the post-cTBS condition had a bigger amount of power along the time, except from 1000 to 2000ms. Overall, the pre-cTBS condition had a higher amount of lower alpha and for the higher alpha, the power was bigger for the post-cTBS condition. The graph for the beta band, after the TMS, we could observe increased over time, except in the first second. The obtained graph was presented as supplementary material on CD (figure A52).

    - **Stroke Patient**

The alpha power between 8-10Hz in the post-cTBS condition increased in all periods, with exception of 1000 to 2000ms. For the higher alpha there was an increase caused by cTBS between -1000 and 1000ms and in the last second, a decrease was also seen between 1000 and 2000ms and remained constant from -2000 to -1000ms and from 2000 to 3000ms. For the beta band we have a higher amount of power after the TMS until 1000ms and then we start having a decrease compared to the pre-cTBS condition. The obtained graph was presented as supplementary material on CD (figure A53).

- **Right Thumb Opposition between 8-10Hz**

    - **Matched-control**

For the matched-control we could see the activation from -1000ms over parietal electrode sites predominantly on the left hemisphere. After 0ms it spreads to the fronto-centro-parietal electrodes. After the 2000ms, the alpha activation started to

decrease. At 2000 to 4000ms we could see a marked deactivation on the surrounding area. After cTBS, the focus was detected over the fronto-centro-parietal electrodes sites on the right hemisphere and it was becoming more expanded and inhibited than the surrounding areas. The left hemisphere was inhibited by the cTBS protocol, so we expected to see an excitation on the contralateral hemisphere. Effectively, we could see an increase of negativity of alpha, which suggests it actually occurred the desired excitation. The obtained topographic maps were presented as supplementary material on CD (figure A54).

- **Stroke Patient**

For the stroke patient we see a positivity in two seconds before the movement onset. From -2000 to -1000ms, the deactivation on the right hemisphere was mainly on central areas, became activated in the following second. This negativity spreads to the left hemisphere over the frontal, central and parietal electrodes sites. Two seconds after the movement onset, we start to see a positivity instead, and the alpha became more deactivated in the following period. After the TMS, the brain's topography showed an activation of alpha, before and during the movement. From 0 to 1000ms there was a clear activation of the alpha on the right hemisphere over whole brain. Following that period, the activation begins to decrease over time. The obtained topographic maps were presented as supplementary material on CD (figure A55).

- **Right Thumb Opposition between 10-12Hz**

  - **Matched-control**

We start to see the same activation over parietal site, and then spreads mainly to the centro-parietal site. After the 1000ms, the negativity started to decrease significantly over the fronto-centro areas and over the parietal sites the negativity stays with similar power. After the cTBS, the brain topography was more negative. The left hemisphere was more inhibited comparatively to the right hemisphere which was more excited. We see the same topography over centro-parietal site as we see for alpha between 8-10Hz. The obtained topographic maps were presented as supplementary material on CD (figure A56).

- **Stroke Patient**

For the stroke patient the topography and the power of the higher alpha was similar to that one described for the lower alpha, before and after the cTBS protocol. The obtained topographic maps were presented as supplementary material on CD (figure A57).

- **Right Thumb Opposition between 15-25Hz**

  - **Matched-control**

Before the movement the brain was more positive. One second before the movement started we see the beta band decreasing, mainly in two focus over the right hemisphere, one over parietal and the other, over frontal electrodes sites. In the following second, this activation spreads to the left hemisphere and central areas. This activation looks to decrease from the 1000 to 2000ms, but in the following two seconds, we could see the two focuses becoming bigger and more negative. After the TMS, the brain showed a clear activation one second before the movement begin. This activation, on both hemispheres over fronto-central-parietal electrodes sites, became larger and stronger. The obtained topographic maps were presented as supplementary material on CD (figure A58).

  - **Stroke Patient**

The beta band was deactivated on the midline over the frontal areas, from -2000 to -1000ms. Then, that focus became less positive. In the surrounding areas the beta band was more activated over the central and parietal areas on both hemispheres. Two seconds after the movement onset, this negativity started to decrease and we began to see again: a focus on the frontal site where the beta band became more deactivated over time. After TMS, the focus that we see on the pre-TMS condition appears in the second before to the movement onset. That focus was more activated, mainly in the following second once the movement onset and 3 to 4 seconds after. The obtained topographic maps were presented as supplementary material on CD (figure A59).

- **Time Frequency: Right Thumb Opposition**

  - **Matched-control**

When the subject began to move the right hand we could see a clear activation on the electrodes C3 and CP3, which disappeared during the movement. Once the movement

stopped, a deactivation on both electrodes approximately from 1400ms could be detected. After the TMS, this deactivation and activation, when the movement began and stopped, was much stronger and explicit. The obtained time-frequency was presented as supplementary material on CD (figure A60 and A61).

- **Stroke Patient**

Before the TMS condition we could see a distinct pattern of activation on the electrodes C3 and CP3 and when the movement ended, the deactivation was seen but it was not very strong. After the TMS, we still see an activation once the movement begins, but was not as strong as we saw before the TMS. It was seen a deactivation during the movement, but this pattern was not seen when the movement stopped. The obtained time-frequency was presented as supplementary material on CD (figure A62 and 63).

- **Quantification Graphs: Right Thumb Opposition**
  - **Matched-control**

The power of alpha between 8-10Hz and 10-12Hz after the TMS was bigger compared to the pre-cTBS condition. For the beta band, we could see over time a bigger power before the TMS. The obtained graph was presented as supplementary material on CD (figure A64).

- **Stroke Patient**

The lower alpha after the TMS showed a decrease until 0ms and then, increased the power up until the end. For the higher alpha we have the same pattern, except in the last second, where we have a bigger power for the pre-cTBS condition. In the first three seconds and from 2000 to 3000ms, the beta band has a similar power in the pre- and post-cTBS condition. We could see that beta after cTBS in the fourth second was bigger and in the last second the beta power was bigger before cTBS condition. The obtained graph was presented as supplementary material on CD (figure A65).

- **Left Thumb Opposition between 8-10Hz**
  - **Matched-control**

For the matched-control we could see a positivity before the movement and, one second before the movement started, we begin to see a negativity. In fact, the deactivated focus observed between -2000 and -1000ms on the right hemisphere over the centro-

parietal sites, became more negative in the next second. The activation of alpha spreads all over the brain and, in the next seconds, we begin to see two focus in both hemisphere on the centro-parietal electrodes sites. These two focus were more positive compared to the surrounding areas which were more negative. When these negativity started to decrease in power and in size, the two focus became more deactivated, mainly on the right hemisphere. After the TMS we saw a negativity in all brain and when the movement begins, we could see a more evident activation focus at centro-parietal sites on the right hemisphere. This focus was observed one second before the movement begins. Then we see over the frontal areas on both hemispheres a negative focus, which was also present from -3000 to -2000ms. The obtained topographic maps were presented as supplementary material on CD (figure A66).

- **Stroke Patient**

For the stroke patient between -2000 and -1000ms we could see an activation of alpha, on the left hemisphere. In the next second, it was seen an activation in the right hemisphere becoming extended to the left one. From 2000 to 3000ms, the negativity became more spread over the fronto-central-parietal areas on both hemispheres. But, this activation of alpha was more marked over central and posterior areas. After the TMS, before imagining the movement, the brain was more positive when compared to before the TMS. When the stroke patient started to imagine the movement, we start seeing a negativity over the fronto-central areas on both hemispheres that become weaker in the following seconds and a negative focus over temporal area on the right hemisphere that remains constant. We also see a deactivation focus after the 0ms, over centro-parietal electrodes on the left hemisphere, which was becoming more positive over time until 3000ms. The obtained topographic maps were presented as supplementary material on CD (figure A67).

- **Left Thumb Opposition between 10-12Hz**

  - **Matched-control**

For the matched-control the topography and alpha power was similar to that one observed for 8-10Hz, before and after the cTBS protocol. The obtained topographic maps were presented as supplementary material on CD (figure A68).

- **Stroke Patient**

For the stroke patient the topography and negativity were similar to what we observed before the TMS for the lower alpha. After the TMS, the topography was also the same, but the power of the negativity observed was not as strong as we see for the alpha between 8-10Hz. The obtained topographic maps were presented as supplementary material on CD (figure A69).

- **Left Thumb Opposition between 15-25Hz**

  - **Matched-control**

For the matched-control we keep seeing a focus on the right hemisphere, which was deactivated before the movement, and in the next second becomes less positive. In the rest of the brain we see the same negativity but was stronger than we see for the lower and higher alpha. After the TMS, we observed again a focus on the centro-parietal sites on the right hemisphere, before the movement begins. After this second and over time, these negativity spreads to the left hemisphere and to the frontal areas. Despite what we see, for the alpha, where the negativity was lateralized to the right hemisphere, for the beta band, the negativity was maintained on both hemispheres. The obtained topographic maps were presented as supplementary material on CD (figure A70).

  - **Stroke Patient**

For the stroke patient, the beta band showed a negative topography. One second before the patient started to imagine the movement, this negativity became to spread for both hemispheres, to the frontal, central and parietal areas. This activation remains affirmative through the time, presenting a maximum power between 2000 and 3000ms. After the TMS, the brain was more positive. Between -2000 and 0ms, it appears an activated focus mainly on the right hemisphere over the frontal sites. From this period, this negative focus became stronger and extends to the left hemisphere over fronto-central areas. However, this negativity observed was not as intense as we saw before the TMS. The obtained topographic maps were presented as supplementary material on CD (figure A71).

- **Time Frequency: Left Thumb Opposition**

  - **Matched-control**

When the movement begins we could see a clear activation around 10-40Hz for electrode C4 and CP4. During the movement that activation was not seen and when the movement stopped, we start to see the deactivation. After the TMS, the activation seen previously, when the subject begins to perform the movement, was not observed so clearly. After the movement stopped, we could observe the deactivation, but was not as strong as we seen before the TMS. The obtained time-frequency was presented as supplementary material on CD (figure A72 and A73).

  - **Stroke Patient**

Before the TMS we see an activation when the patient to perform the task, but this activation was stronger after the inhibitory protocol. When he stopped to imagine the movement, surprisingly it was seen an activation instead of a deactivation in the first second, in the pre-cTBS condition. Though, the deactivation pattern was seen after the TMS, mainly over the electrode C4. The obtained time-frequency was presented as supplementary material on CD (figure A74 and A75).

- **Quantification Graphs: Left Thumb Opposition**

  - **Matched-control**

The lower alpha before the TMS was bigger from -2000 to -1000ms, 0 to 1000ms and 3000 to 4000ms. Between these periods, cTBS induced a bigger power for alpha. The higher alpha had a similar behavior to the lower alpha. This relation was not observed for the beta band, because we have a lower beta power after the cTBS protocol, comparing with the beta before cTBS. The obtained topographic maps were presented as supplementary material on CD (figure A76).

  - **Stroke Patient**

The power of alpha between 8-10Hz before the TMS was bigger from -2000 to -1000ms, 0-2000ms and in the last second. Overall, the alpha before the TMS had more power. The higher alpha before the TMS was bigger from -2000 to -1000ms and from 0 to 2000ms. The beta band was bigger before the inhibitory protocol between -1000 and

2000ms and 3000 to 4000ms. So, for the beta band we have more power before the TMS. The obtained topographic maps were presented as supplementary material on CD (figure A77).

- **Both Thumb Opposition between 8-10Hz**
    - **Matched-control**

For both thumb opposition between 8-10Hz, we could visualize two main focuses, one over the centro-parietal areas and the other over the frontal electrodes sites on the right hemisphere between -2000 and -1000ms. Also, a smaller focus was seen on the left hemisphere over centro-parietal areas in the same time period. We see a diminution of the alpha power between -1000 and 0ms, but after that period we see a deactivation of alpha on the centro-parietal area on both hemispheres, mainly on the left hemisphere. After the cTBS the brain exhibits a negative topography. We see the same focus on the centro-parietal areas on both hemispheres between -1000 and 1000ms. After the 1000ms the deactivation started to increase mainly on the right hemisphere until the 2000ms, and in the following seconds, the negative pattern returns. The obtained topographic maps were presented as supplementary material on CD (figure A78).

- **Stroke Subject**

The stroke patient showed a negativity in almost all brain between -2000 and -1000ms, and this activation increases over the fronto-central electrodes on both hemispheres, in the next second. The topography maintains over the time and the activation started to decrease. In the last second, 3000 and 4000ms, we see a negativity increased on the whole brain. After the cTBS protocol, the topography showed to be more positive comparing to the pre-cTBS condition. Between -2000 and -1000ms, over the fronto-central-parietal electrodes on both hemispheres, we see a deactivation of the lower alpha, which was not seen before the protocol. In the following seconds, this deactivation became more lateralized to the right hemisphere on the frontal and central electrodes but not on the parietal. In the last second it seems that the negativity was decreasing in the left hemisphere and the positivity was increasing. The obtained topographic maps were presented as supplementary material on CD (figure A79).

- **Both Thumb Opposition between 10-12Hz**

    - **Matched-control**

The higher alpha showed a similar topography and distribution as we seen for the lower alpha between -3000ms and -4000ms. After the inhibited protocol, we see again the same topography over the centro-parietal areas, but the activation seems to be stronger than the alpha between 8-10Hz. The obtained topographic maps were presented as supplementary material on CD (figure A80).

    - **Stroke Patient**

For the higher alpha this band was supposed to have a central topography. But, we see a focus mainly on the parietal sites over the left hemisphere which appears from -1000ms, where the activation was increasing over time, except between 2000 and 3000ms. Between -1000 and 0ms the activation was seen on the right hemisphere and from 3000 to 4000ms it was observed on both hemispheres and became more spread, over the fronto-central sites. After the protocol was used, between -1000 and 0ms, we begin to see an activation on both hemispheres, on the fronto-centro-parietal electrodes, comparing with the second before. The negativity started to increase and spreads until we reach the 2000ms, and then started to decrease in the following two seconds. The obtained topographic maps were presented as supplementary material on CD (figure A81).

- **Both Thumb Opposition between 15-25Hz**

    - **Matched-control**

The beta band showed bigger deactivation on fronto-centro-parietal electrodes over both hemispheres comparing with the surrounding areas. This deactivation decrease significantly between -1000ms and 1000ms and then, maintains similar over time and the topography was also the same. The focus on the right hemisphere was not dominant as we seen for the alpha band. After the cTBS protocol the beta band showed a negative power after the -2000ms. This negativity increases significantly after 0ms in whole brain and was constant over time. The obtained topographic maps were presented as supplementary material on CD (figure A82).

    - **Stroke Patient**

The stroke subject showed an activation between -3000 and -2000ms. This negativity was seen on both hemispheres, but the topography showed to be greater on the

left hemisphere. The negative focus started to be more positive over time until 3000ms; the positivity that was seen was also greater on the left hemisphere. After the cTBS protocol, the positivity saw mainly on the left hemisphere was now replaced for a negativity. The activation spreads for the right hemisphere and we see a brain with a clearly negative topography during movement. The obtained topographic maps were presented as supplementary material on CD (figure A83).

- **Time Frequency: Both Thumb Opposition**

    - **Matched-control**

The time-frequency reveals an activation on the alpha band for the electrode CZ when the subject begins the movement, comparing with the baseline before movement. When the subject stopped to moving the hands, we see a deactivation over the electrode C3, CZ and C4 approximately between 8Hz to 28Hz. After the cTBS protocol, principally, over the electrode C3 and C4 we see an activation when the subject begins to perform the movement. We also see for the electrode CZ but the power was lower compared with the electrode C3 and C4. During the movement this activation was not seen. When the movement stopped we see a deactivation for the electrode C3, CZ and C4. This deactivation was seen mainly over the electrode C3. Comparing before and after cTBS, the activation was greater after the inhibiting protocol when the subjects begins the motor tasks, and the deactivation was bigger for the electrode C3 and lower for the electrode C4 and CZ . The obtained time-frequency was presented as supplementary material on CD (figure A84 and A85).

    - **Stroke Patient**

The time frequency did not reveal a pattern. Over the electrode C3 we see a deactivation greater before movement than when the patient started to perform the movement. This may be due to an over-activation of the non-injured hemisphere. After the movement, the deactivation was lower on C3, CZ and C4 comparing when the subject began to perform the movement. Though, after the cTBS we could see a clear pattern of activation when the patient begins to perform the movement on all the selected electrodes and this negativity decrease significantly when he stopped the movement. The obtained time-frequency was presented as supplementary material on CD (figure A86 and A87).

- **Quantification Graphs: Both Thumb Opposition**

    - **Matched-control**

In the post-cTBS condition, the lower alpha had a higher amount of power only between -1000 and 0ms. Thus, we have more alpha in the pre-cTBS condition. After the cTBS, the higher alpha had more power until reaches the 0ms and after that period we have less alpha compared to the pre-cTBS condition. The beta band after the cTBS protocol was higher until it reaches the 0ms and from 1000 to 2000ms. Between 0 and 1000ms we cannot see the difference between both conditions and in the last two seconds there was a decrease after the cTBS. The obtained graph was presented as supplementary material on CD (figure A88).

    - **Stroke Patient**

For the lower alpha the amount of power in the first two seconds and in the last second was bigger after the TMS. In the following seconds the pre-cTBS condition had more alpha until 3000ms. This was also seen for the alpha between 10-12Hz. The beta band had also less power in the first two seconds before the inhibitory protocol, and in the following seconds, the power was bigger pre-cTBS. So, we have more beta power before the TMS. The obtained graph was presented as supplementary material on CD (figure A89).

As it was indicated, we show the main results for the patient and matched-control for the brain's topography on table 6. In the summary table of the quantification graphs (table 7) we also included the observations for the control group that was stimulated on the left hemisphere to facilitate interpretations and discussion of the results. When the lower and higher alpha showed the same tendency, we designated both as alpha.

Summary Table Brain's Topography		
Tasks	**Effects of cTBS for the matched-control on the left hemisphere**	**Effects of cTBS for the stroke patient on the left hemisphere**
Eyes close	Alpha ↓	Alpha ↓
Right Arm	Alpha ↓ Beta ↓	Alpha ↓ Beta ↓
Left Arm	Alpha ↓ Beta ↓	Alpha ↑ Beta ↑
Both Arm	Alpha ↓ Beta ↑	Alpha ↑ Beta ↑
Right Hand	Alpha ↓ Beta ↓	Alpha ↓ Beta ↓
Left Hand	Alpha ↑ Beta ↑	Alpha ↑ Beta ↑
Both Hand	Alpha ↓ Beta ↓	Lower Alpha ↑ Higher Alpha ↓ Beta ↓

Table 6. Summary table of brain's topography for the matched-control stimulated on the left hemisphere and the stroke patient stimulated on the left hemisphere. The results represent the variation on alpha and beta power induced by the cTBS protocol.

Summary Table Quantification Graphs			
Tasks	**cTBS protocol is applied on the control group on the left hemisphere**	**cTBS protocol is applied on matched-control on the left hemisphere**	**cTBS protocol is applied on the stroke patient on the left hemisphere**
Eyes close	Alpha ↓	Alpha ↓	Alpha ↓
Right Arm	Alpha ↑ Beta ↓	Alpha ↑ Beta ↑	Alpha ↓ Beta ↓
Left Arm	Alpha ↑ Beta ↑	Alpha ↑ Beta ↑	Alpha ↓ Beta ↓
Both Arm	Alpha ↓ Beta ↓	Lower alpha ↓ Higher Alpha ↑ Beta ↑	Alpha ↑ Beta ↓
Right Hand	Alpha ↑ Beta ↑	Alpha ↑ Beta ↓	Alpha ↑ Beta ≈
Left Hand	Alpha ↑ Beta ↓	Alpha ↑ Beta ↓	Alpha ↓ Beta ↓
Both Hand	Alpha ↑ Beta ↓	Alpha ↓ Beta ↑	Alpha ↓ Beta ↓

Table 7. Summary table for the quantification graphs for the control group, the matched-control and the stroke patient, all stimulated on the left hemisphere. The results represent the variation on alpha and beta power induced by the cTBS protocol.

6. DISCUSSION OF RESULTS

First we are going to discuss the results for both control groups, and then, for the matched-control and the stroke patient. To a better discussion we believe it is better to understand what happens in normal conditions, i.e. in health, and then to evaluate the differences between a healthy subject and a stroke patient before and after the cTBS protocol.

6.1 Discussion of results for the controls

Knowing that the right-handedness represents the brain function lateralization, the controls were divided in two groups: 5 subjects were stimulated in the right hemisphere (non-dominant) and the other 6 subjects were stimulated in the left (dominant) hemisphere. The subjects that were stimulated with the cTBS protocol in the right hemisphere was supposed to have the brain activity inhibited in that hemisphere, and in the contralateral hemisphere should be increased. When the inhibitory protocol was applied in the left hemisphere, this hemisphere should be inhibited and, consequently, the right hemisphere should be more excited.

It was not found studies to know if the cTBS protocol when is applied on the dominant or non-dominant hemisphere can affected the motor biomarkers (alpha and beta rhythms) differently. We only found a study with healthy subjects that received low frequencies rTMS, the authors reported that when this protocol is applied on the dominant hemisphere M1 it improves the ipsilateral hand function, but when it is applied on the non-dominant it is not seen significant influence over ipsilateral or contralateral manual dexterity (Weiler et al., 2008). This suggests that there can be an influence of the dominance of the hemisphere on the response to some TMS protocols.

Eyes closed

When the eyes were closed the group that was stimulated in the right hemisphere had an increase of alpha after cTBS compared to the pre-cTBS condition while the group that was stimulated in the left hemisphere had a decrease of alpha. So, the brain became deactivated for the first group, as it was expected, while for the other group, the brain was more activated. This difference of results corroborates our hypothesis that the cTBS protocol affects differently the two hemispheres which is accordance to Weiler *et al*. (2008). Based on this observation, we could suppose that when cTBS is applied to the

non-dominant side there would be a deactivation; on the other hand, applying cTBS to the dominant hemisphere we would observe an activation.

Right arm elevation

After cTBS is applied to the right hemisphere it is thought to occur an inhibition on the right hemisphere and an excitation on the left hemisphere. When the subjects raise the right arm after the protocol, it is supposed to be observed an activation on the left hemisphere, translated into a decrease on the alpha and beta power of the left side electrodes. However, in the post-cTBS right arm elevation, the obtained result was opposite to what was expected, showing an increase for the lower and higher alpha as well as for the beta. For the other group, which was stimulated on the left hemisphere, the inhibition should occur on the left hemisphere, associated to an excitation on the right hemisphere. Therefore, we should see an alpha and beta power increase on the left electrodes. Although for the beta power the results were contradictory to this theory, for the lower and higher alpha power we obtained the expected increase after the right arm elevation post-cTBS.

Left arm elevation

On the group that received cTBS on the right hemisphere, when the subjects raise the left arm after the protocol, it is supposed to be observed a deactivation on the right hemisphere, translated into an increase on the alpha and beta power of the right side electrodes. On the contrary, when the protocol was applied to the left hemisphere, we should observe an activation on the right hemisphere associated to an alpha and beta decrease. In this motor task the group that was stimulated on the right hemisphere showed results consistent with the estimated; on the other group the power was supposed to decrease but, instead, it increased for all frequencies.

Both arms elevation

When the subjects elevated both arms, the brain was more deactivated for the group that received the cTBS on the right hemisphere and in the other group, the brain was more activated. This was already described when the two groups were with eyes closed and in accordance with our hypothesis that with cTBS applied to the non-dominant side there is a deactivation whereas when the cTBS is applied to the dominant hemisphere we observe an activation.

Right hand opposition

For the right hand opposition, in the group stimulated in the left hemisphere the graph shows a bigger increase of power mainly for the lower and higher alpha, and is also seen for the beta band. This was expectable because in this group the brain activity on the left hemisphere was more inhibited, so it was supposed to have a bigger amount of alpha and beta compared to the pre-cTBS condition. On the other group, overall, the power for alpha frequencies, decreased as it was supposed because the left hemisphere was over activated due to the right hemisphere inhibition.

Left hand opposition

On the next motor task, left hand opposition, we obtained an increase of power for both the alpha and beta bands when the cTBS protocol was applied to the right hemisphere. These results were in accordance to our hypothesis that applying cTBS to the right hemisphere, we would have an inhibition on this hemisphere accompanied by an alpha and beta increase. For the other group, where the cTBS was applied on the left hemisphere, the alpha frequencies in study showed also a power increase for the post-cTBS condition, though we expected a decrease of alpha power related to an excitation on the right hemisphere. The beta band showed a decrease for this group after cTBS as it was supposed.

Both hands opposition

For the last motor task, both hands opposition, when the cTBS protocol was applied to the non-dominant hemisphere it increased always the beta power independently from the movement performed. On the other hand, for the dominant hemisphere that was not verified since the beta power varied according to the type of movement. In this group, beta behavior was more consistent with the expected when performing more precise movements, i.e. finger opposition.

Analyzing the table 5, when we applied the cTBS protocol to the non-dominant hemisphere the alpha power remained always increased for less complex movements, such as arm elevation, showing the same behavior as beta. For finger opposition tasks, alpha behavior seemed to correlate to the movement. The application of cTBS protocol to the dominant hemisphere resulted on changes of alpha power for the arm elevation task. So, the right and the left arm elevation led to an increase of the alpha power, while the both arms elevation showed a decrease. For the finger opposition the alpha always

increase independently from the movement. This way, the application of cTBS to the dominant hemisphere demonstrated that mainly the beta band was influenced by the motor task. The alpha band was not dependent on the motor task, except for the both arms elevation, while the beta band was more relate to the fine movements. The stimulation of the non-dominant hemisphere with the inhibitory protocol resulted on alpha variation associated to the task for the more complex movements and the beta band increased after the cTBS and this was not dependent on the motor task.

There were no relevant differences between lower and higher alpha on every tasks, for both groups analysis.

6.2 Discussion of results for the matched-control and stroke patient

We only had one stroke patient, and therefore, we used one control to compare the results of topographic maps and time frequency. The cTBS protocol on both cases was applied on the left hemisphere. Additionally, we compared the quantification graphs between both subjects (patient and matched-control) and the whole group stimulated on the left hemisphere. Thus, this hemisphere was supposed to be more inhibited after cTBS, so we should have more alpha and beta power, and the right hemisphere was supposed to be more excited, therefore, we should have less alpha and beta power. We applied it on the left hemisphere in the stroke patient to try to decrease the activity on the non-lesioned hemisphere which was over activated and, consequently, increase the activity on the lesioned (right) hemisphere due to interhemispheric connections (figure 27).

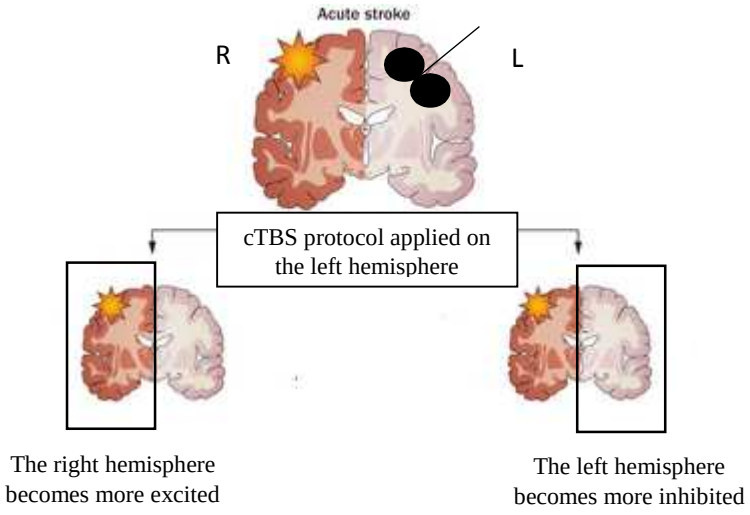


Figure 27. Schematic illustration of the effects when the cTBS protocol was applied on the left hemisphere to the stroke patient.

Eyes closed

When the eyes were closed, on the topographic maps and on the quantification graphs, we saw a decrease on alpha power after cTBS protocol for both the patient and the control. Therefore, the brain became more activated. These results were similar to those obtained for all the controls stimulated on the left hemisphere. We hypothesized that when cTBS is applied to the dominant hemisphere it induces an activation on the brain's topography as we could see on the topographic maps for the patient and matched-control.

For the motor tasks, three different types of event-related ERD/ERS patterns at the scalp EEG have been mainly described, which are:

1. ERD in the mu and beta band about 2 seconds before the movement onset over contralateral sensorimotor areas;
2. Alpha and beta ERD spreads symmetrical and bilaterally with the movement initiation;
3. Within the first second after the movement offset is seen a contralateral dominant beta rebound (beta ERS), while mu rhythm is still seen with a desynchronized pattern.

Right arm elevation

Before the stimulation, for the matched-control, on the first motor task, we did not see a marked event-related ERD. This motor task, for the stroke patient, showed a focus of activation on the centro-parietal areas for all frequencies in study and this focus spread bilaterally. According to Amengual *et al*. (2014) stroke patients recruit the same areas of the brain for simple motor commands as if it was a more complex task. This can be the reason why we saw a focus of ERD for the stroke patient but not for the matched-control.

After cTBS protocol, for right arm elevation, the quantification graphs showed an increase on alpha (higher and lower) and beta power for the matched-control, like it was supposed. However, for the stroke patient there was a decrease on alpha and beta power.

Left arm elevation

For the following task, left arm elevation, observing the pre-cTBS condition, the non-dominant hemisphere of the matched-control presented an activation between -2000 and 0ms followed by a deactivation. Despite what we saw for the right arm, for the left arm the focus became bilateral with the movement initiation, as it was expected. The left

arm showed a bigger ERD focus compared to the right arm task, which is in agreement for what has been described by Fu *et al.* (2006). After the TMS, the brain's topography was more negative, therefore, more activated. For the stroke patient, before the cTBS we saw an activation pattern for all frequencies in study, on both the affected and unaffected hemispheres, when he imagined to elevate the left arm. This was already described by Scherer *et al.* (2007). The ERD found for the patient, due to its dimensions and topography, can be associated to the higher impairment and spasticity according to Kaiser *et al*. (2012). After the cTBS protocol, the activation seen previously was reduced mainly for the higher alpha and beta band.

Along with left arm elevation post-cTBS, on the graphs we observed an increase of alpha and beta power for the matched-control as it was noticed for the group analysis, although this effect was contrary to what we expected. For the stroke patient, on the other hand, we saw the expectable decrease in all frequencies after the application of the protocol. Nevertheless, since he imagined the movement, and we saw the same pattern when he lifted the right arm, we cannot assure that this decrease was directly correlated to the task performed. In this motor task, the cTBS protocol seemed to also have a different effect on the patient compared to the controls.

Both arms elevation

Analyzing the both arms elevation for the matched-control, the cTBS protocol diminished the alpha power for the lower and higher frequencies, while for the beta band we saw an increased power. For the first time, we saw a different effect for the alpha and for the beta band after the cTBS, which was already described by Shafi *et al.* (2014). For the stroke patient, this motor task showed a similar ERD pattern and the topography was the same as we saw for the left arm. We can hypothesize that when the patient imagined a movement the ERD was stronger enough to spread for all brain. After the TMS, the lower and higher alpha and the beta band became more positive in comparison to the pre-cTBS condition.

On the quantification graphs we saw for the matched-control a decrease of power for the lower alpha after the TMS but, at the same time, an increase for the higher alpha and beta bands. This was the first task demonstrating a difference between higher and lower alpha response to cTBS. Interestingly, the group analysis revealed a decrease of power after cTBS, mainly for the higher alpha and beta power. For the stroke patient, there was an increase of both alpha bands; therefore, we did not see a different pattern

between lower and higher alpha, as we saw for the matched-control. Despite what we visualized for the alpha band, the beta power decreased after TMS. Again, the cTBS protocol influenced differently the stroke patient, compared to the matched-control, except for the higher alpha behavior.

The three motor tasks performed with arm(s) elevation did not show the expected event-related ERD/ERS patterns at the scalp EEG, mainly for the matched-control. This was probably due to this tasks not being complex enough for the healthy subject to induce the patterns. The number of repetitions could also not be sufficient to show the patterns for the mu and beta band.

Right hand opposition

For the matched-control, in the fourth motor task (right thumb opposition), before the cTBS protocol we could see a focus of activation at central and parietal electrodes sites mainly for the lower and higher alpha. For these frequencies, during the movement we saw a deactivated focus near an activated focus. What we saw may have been the "focal ERD/surround ERS" which had been described by several authors, such as, Neuper & Pfurtscheller, (2001). As it was seen for the lower and higher alpha this was not as specific for the higher alpha as it was described by Ramos-Murguialday & Birbaumer, (2015). These two focus showed an opposite power after the TMS. For the beta band we did not see the "focal ERD/surround ERS", but it was seen a spread of the beta band with the movement initiation as it was illustrated by Ramos-Murguialday & Birbaumer, (2015) and McFarland *et al.* (2000). After the TMS, the brain was more activated for all frequencies and on the time-frequency we saw a beta rebound within the first 500ms after the movement offset, which follows what Neuper *et al.* (2006) had already described. For the stroke patient, on the pre-cTBS condition, it was observed an activated focus in the first second before the movement onset on the affected hemisphere for the lower and higher alpha. For the beta band, we saw two focus on both hemispheres over centro-parietal electrodes sites.

On the quantification graphs, associated to the right hand opposition, it was supposed to occur an increase on alpha and beta power after the TMS. The increase on alpha was observed for both the patient and the matched-control. However, analyzing the beta band, we saw a different response between the matched-control and the

corresponding group. In this task, the patient showed a similar effect of cTBS for alpha power but not for beta, when comparing to the healthy individuals.

Left hand opposition

For the matched-control, in the left hand opposition it was seen a strong activation for the mu and beta bands, before the TMS. Comparing the movements with the right hand *versus* the left hand we did see bigger hemispheric asymmetries for the right hand and this was in accordance with McFarland *et al.* (2000). Also, we did see a bigger activation for the non-dominant hand comparing to the dominant hand, as Fu *et al*. (2006) described. For the stroke patient we saw a desynchronization on the left hemisphere and this was already described by Scherer *et al.* (2007). The large activation saw on the contralateral hemisphere, according to Kaiser *et al.* (2012), was probably due to the higher impairment of the patient. The brain topography after the TMS for the stroke patient showed the same decrease in the negativity that we had seen to the left arm.

In this motor task, it was expected a decrease of alpha and beta power on the quantification graphs following the cTBS protocol. However, analyzing the matched-control alone and the whole group that received cTBS on the left hemisphere, we had an increase of lower and higher alpha and a decrease of beta power. The stroke patient revealed a different pattern for alpha, showing a decrease of power, corresponding to what was described above as being expected.

Both hands opposition

In the last motor task, both thumb opposition, McFarland *et al.* (2000) described that we should see two main focus of desynchronization for the mu which should be stronger on the left side of the brain. On the contrary, for the lower and higher alpha, we saw two main focus of synchronization. The topography for the alpha band was in accordance with McFarland *et al.* (2000) findings since during movement preparation the focus was bigger on the right hemisphere, but over time it became stronger on the left hemisphere. For the beta band it was seen a diffuse topography, two seconds before the movement, which was also in accordance by McFarland *et al.* (2000). After the TMS, the brain's topography was highly activated for the frequencies in study, mainly for the beta band. For the stroke patient, on the pre-cTBS condition, mainly the lower and higher alpha showed an activation pattern on the brain's topography. In this motor task, when the patient was performing the movement for the right hand and imagining for the left hand, the excitability of the motor neurons was reduced only for the alpha band. This

motor task did not show a bigger ERD which could be explained by the subject not being imagining the movement. After the TMS, the lower alpha became more positive and the higher alpha and the beta band became more deactivated.

When the matched-control and the stroke patient did the both hands opposition, we saw in box and whiskers plots a decrease of lower and higher alpha after cTBS, contrary to what was observed for the whole group analysis. cTBS decreased the beta power for the stroke patient and the control group, although for the matched-control there was a beta power increased.

Comparing table 6 and table 7, for the motor tasks we can observe some differences between the quantification graphs and the brain's topography results, associated to the different methodologies. We assume that this happened because for the brain's topography we had 62 electrodes selected while for the quantification graphs we selected 7 electrodes of interest for the tasks performed with only one upper-limb and 13 for the tasks performed with both upper-limbs.

According to the results described in section 6.1 and 6.2, some of the observations were different from what we supposed to have. As Hamada *et al.* (2013) reported in their study, some of the subjects could have been excited instead of being inhibited on the hemisphere where we applied the cTBS protocol. The authors also assumed that this fact could be explained by differences in the recruitment of cortical neurons which was observed when the MEP's latency was analyzed. Ilmoniemi *et al.* (2010) and Hamada *et al*., (2013) also described some variability associated to the orientation and location of the coil, the state of the cortex and the vigilance of the subjects, which are also important factors that can affect the EEG response. So, between the matched-control and the subjects of the group, the results were not always similar probably due to the inter-individual variability on the response to the TMS technique. For the motor tasks, between the matched-control and the stroke patient there were often seen different results for the frequencies in study. This is almost certainly explained by the impairments due to the stroke, although we cannot exclude the influence of inter-subject variability.

Ramos-Murguialday & Birbaumer, (2015) stated that it was described in the literature a different topography for the lower and higher alpha, but in our results we could not see this different topography, which can be due to our sample size.

In this study we chose only active movements because according to Park *et al.* (2014) there is bigger desynchronization for active movements than for passive movements. And we saw that movements with hands induced more deactivation and activation for the alpha and beta bands than the movements with the arms.

7. LIMITATIONS OF THE STUDY

The research studies in humans have different limitations. It took a couple of months to design this study, because there were many concerns to take into account and due to the patients being in acute/subacute phase the procedures were reviewed in order to not disturb the patient's condition.

The recruitment of patients only started in February and our inclusion criteria was very limited to homogenize our sample. Due to these reasons the number of subjects that could be included in the study was highly affected. Subjects who met most the criteria had to be excluded mainly due to their clinical situation. These were the main causes by which we only had one patient who participated in the study.

Part of the results obtained for the patient and the matched-control are not in agreement for what has been described in the literature which can be due to the small number of subjects used. The protocol for motor tasks should have more trials to have better results. But, as we had a limited time after the cTBS protocol, the maximum we were able to do was only 6 repetitions for movements.

The onset of the motor task, was defined when I gave the order, but sometimes, the subjects performed the motor task immediately and other times, took a while to perform. Therefore, this is the reason why sometimes, we see a deactivation happening one or two seconds before the onset movement.

One of the limitations was the impossibility to perform an EEG one and/or two months after cTBS session in the patients. It could give us valuable information about the brain’s physiology and its evolution. However, we had to decide only after 3 months, because it was when the patients have an appointment at CHUC.

The control recruitment was also very difficult considering the age of the subjects. The number of hours for one session was also a reason for which many subjects did not want to participate.

After processing the data, the scale used in the Quantification graphs sometimes did not allow us to easily see the statistical significant changes, obtained through the Wilcoxon test, between the two conditions, pre and post-cTBS condition.

8. FUTURE WORK

In the next month we are going to do the follow-up study to the stroke patient, but the sample is too small to achieve significant conclusions. So, the main objective in the future is to have a bigger number of stroke patients to achieve a repost to the following objectives:

- In patients, 3 months after the stroke, we can assess if there was some improvement in the motor biomarkers and if they were linked to previous alterations in the first session;
- Compare the results between patients based on the lesions location to evaluate which one may benefit the most from the cTBS;
- Compare the patients who were able to execute or just imagine the movement to assess if there were brain differences between these subjects after cTBS session;
- Evaluation of desynchronization will be inter-individually and intra-individually to analyze if there is a correlation between patients in the evolution of motor deficits in a 3 months period;
- Analyze if the results can help to ensure a safe method to use as a prognostic measure about recovery ability and able to offer a guide in the path to build personalized rehabilitation treatments.

With these results, we can perform this study with the same protocol in chronic study patients in the Rehabilitation Hospital Rovisco Pais. At this hospital we are going to apply 10 sessions of cTBS, to act as treatment rehab.

The motor tasks for the healthy subjects can be modified. Instead of each movement lasts 15 seconds, if it lasts 5 seconds we could increase the number of our trials and therefore, we may see a better event-related ERD/ERS patterns at the scalp EEG. For the stroke patients will be assessed if the duration of each trail can be changed as well.

For the control group we are going to make a further analysis to analyze if the differences that we observed for the dominant and the non-dominant hemisphere are not caused by an excitement instead of being inhibited on the hemisphere where we applied the cTBS protocol.

9. CONCLUSION

The results in our study showed significant differences when the healthy subjects received the cTBS protocol on the dominant and non-dominant hemisphere. The matched-control and the stroke patient, which received the inhibitory protocol on the left hemisphere, also showed the differences between pre and post-cTBS. Moreover, they presented similar results to those obtained for all the controls stimulated on the left hemisphere. Taking into account our results, the following scheme, figure 28, represents our conclusions for our sample:

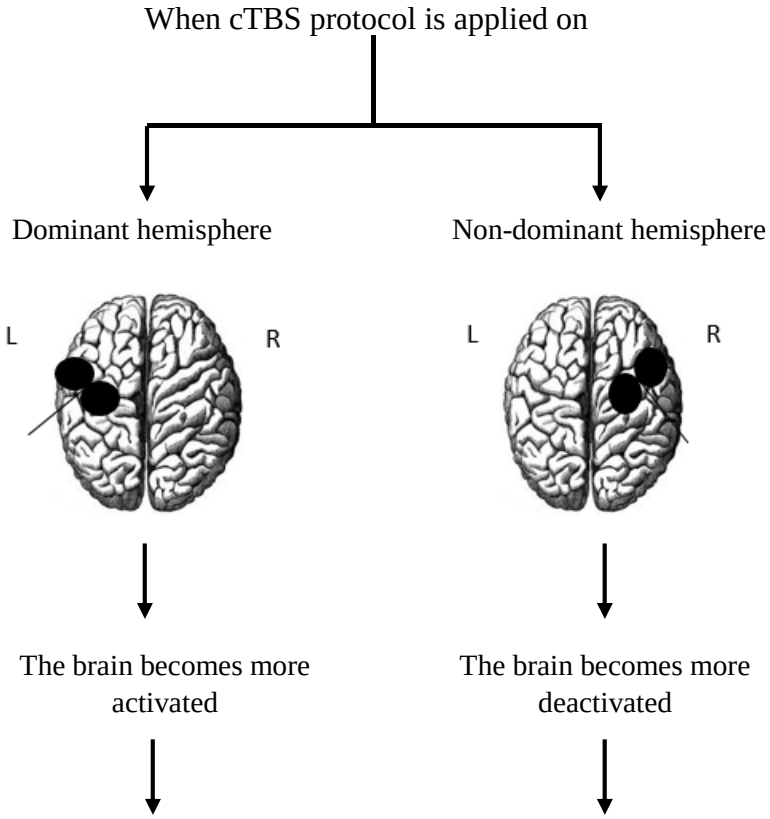


Figure 28. Effects of cTBS when it is applied on the dominant and non-dominant hemisphere.

On the motor tasks for both control groups, we saw that for the group that received the cTBS on the non-dominant hemisphere, the alpha changes with more complex movements and for the other group, the beta band was more influenced by the same type of movements.

For the matched-control and the stroke patient, on the brain's topography, the motor tasks showed that the cTBS had a different effect for the arms tasks, except for the right arm elevation task. Interestingly, for the hand opposition tasks, the effects after cTBS were the same for both subjects. We hypothesized, based on Amengual *et al.* (2014) study that this could have occurred because arm elevation on the healthy subject did not activate the same brain areas as for the stroke patient. For the patient this task was more difficult than for the matched-control; therefore, he recruited different areas of the brain as it was a demanding task. So, the cTBS had a different effect. For the hands tasks, both subjects showed the same effect after the inhibitory protocol, which can be related to the complexity of the task. In fact, this task was more complex either to the healthy individual as to the patient. So, the same brain areas were recruited.

As conclusion, this inhibitory protocol changes the brain's physiology and this was observed when the subjects had the eyes closed. The motor biomarkers (mu and beta band) were affected by the cTBS protocol for all motor tasks. It was also seen that fingers opposition task affected more the mu and beta rhythms compared to the arm(s) elevation tasks. Therefore, the patterns of ERD/ERS were better seen for more complex movements.

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APPENDIX I – Admission form

THE ROLE OF EEG AS A BIOMARKER TOOL IN ASSESSING PLASTIC CHANGES INDUCED BY TRANSCRANIAL MAGNETIC STIMULATION IN STROKE PATIENTS

Admission date: __/__/____

Name:_____ Subject ID:____

☐ Male ☐ Female DOB: __/__/____ Age at admission: __

Relevant health problems history:_____

_____

_____

Family history:_____

_____

_____

Medication (with dosages):_____

_____

_____

Patient's state:_____

_____

Stroke Time since stroke: _____

Imagiologic exams (with results):_____

_____

_____

Lesion characterization (classification & localization): _____

_____

Neurologic deficits:_____

_____

_____

Affected hemisphere: ☐ left ☐ right / ☐ dominant ☐ non dominant

Observations:_____

_____

_____

Conventional rehabilitation treatment:_____

_____

Experiment

Inclusion Criteria	**Yes**	**No**
1. Aged between 18 and 80 years		
2. Poststroke period 7 ± 2 days		
3. First-ever MCA stroke		
4. Cortico-subcortical stroke		
5. Upper limb motor deficit LEVEL_____		
6. Ability to understand the tasks		
7. Modified rankin scale pre- stroke ≤ 1		

Exclusion Criteria	Yes	No
1. Cognitive impairment		
2. Dementia previously documented		
3. History of epilepsy		
4. Neglect		
5. Posterior or global aphasia		
6. Hemiplegia		
7. Pregnancy		
8. Drug and alcohol abuse		
9. Intracranial metallic implant		
10. Artificial cochlear implant		
11. Implanted pacemakers or medication pump		
13. Other		

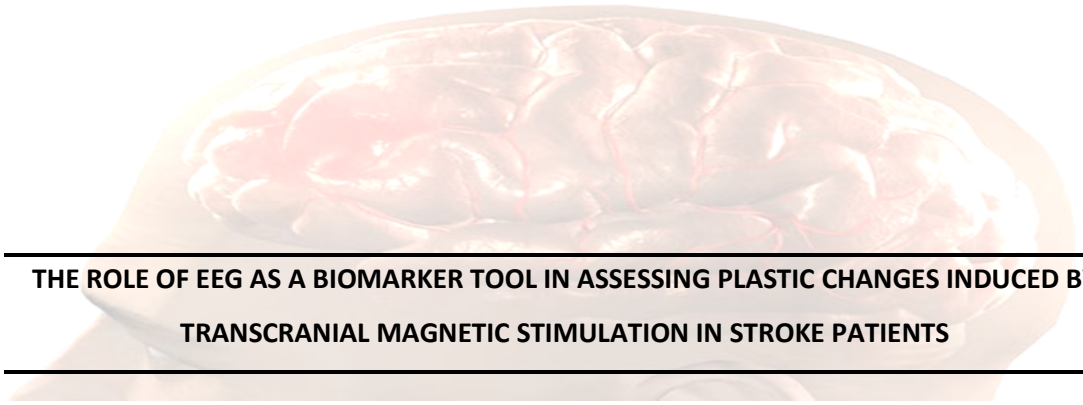
Modified Rankin Scale (Date, result)	_____	_____	NIHSS (Date, result)	_____	_____
	_____	_____		_____	_____

APPENDIX II – Subjects did not join the study

THE ROLE OF EEG AS A BIOMARKER TOOL IN ASSESSING PLASTIC CHANGES INDUCED BY TRANSCRANIAL MAGNETIC STIMULATION IN STROKE PATIENTS

Number of patients	Sex	Age	Reason not to join the study
1	M	47	Not comfortable with machines.
2	M	75	Respiratory infection the day before the exam.
3	M	72	Transferred to Figueira da Foz before completing the 5 days.
4	M	79	Lack of collaboration to participate and including the treatment needed in hospital.
5	M	79	Lack of collaboration to participate and including the treatment needed in hospital.
6	F	68	Family did not agree with their participation.
7	M	80	Excluded due to his clinical history
8	M	68	Did not have interest to participate.
9	M	70	Respiratory infection the day before the exam.
10	F	79	Respiratory infection the day before the exam (plegia, fever)
11	M	72	Respiratory infection.
12	F	76	Coma (imminent cerebral death)
13	F	78	None collaboration to participate.
14	F	72	Went to ICU.
15	M	41	Transferred to Aveiro before completing the 5 days.
16	M	56	Transferred to Porto before completing the 5 days.
17	F	70	Transferred to Leiria before completing the 5 days.

APPENDIX III – Clinical report form for stroke patients



THE ROLE OF EEG AS A BIOMARKER TOOL IN ASSESSING PLASTIC CHANGES INDUCED BY TRANSCRANIAL MAGNETIC STIMULATION IN STROKE PATIENTS

Admission date: __/__/____ Time since stroke: _____ Follow-up date: __/__/___

Name:_____ Subject ID:____

Education level:_____ Job:_____

Address: _____

Phone number: _____

Edinburgh Handedness Inventory: _____

Structural MRI results: _____

_____

Wolf Motor Function Test:

Performance time before_____ after_____ 3 months follow-up: _____

Functional ability score before_____ after_____ 3 months follow-up: _____

EEG observations: _____

_____

TMS ☐ real ☐ sham First time doing TMS? ☐ Yes ☐No

UNAFFECTED HEMISPHERE

Muscle _____

Before cTBS MT _____ **3 months** MT _____

Before cTBS rMT _____ **After cTBS** rMT _____ **3 months** rMT _____

Active MT _____ 80% active MT _____

AFFECTED HEMISPHERE

Mean of MEP test/mean of MEP conditioning

Before	Affected	**After**	Affected
MT	_____	MT	_____
rMT	_____	rMT	_____
SICI (1/3/5 ms)	___/___/___	SICI	___/___/___
ICF (10/15/20 ms)	___/___/___	ICF	___/___/___
LICI (50/100/150 ms)	___/___/___	LICI	___/___/___

3 months	Affected
MT	_____
rMT	_____
SICI	___/___/___
ICF	___/___/___

Side effects: _____

_____

AFTER SESSION

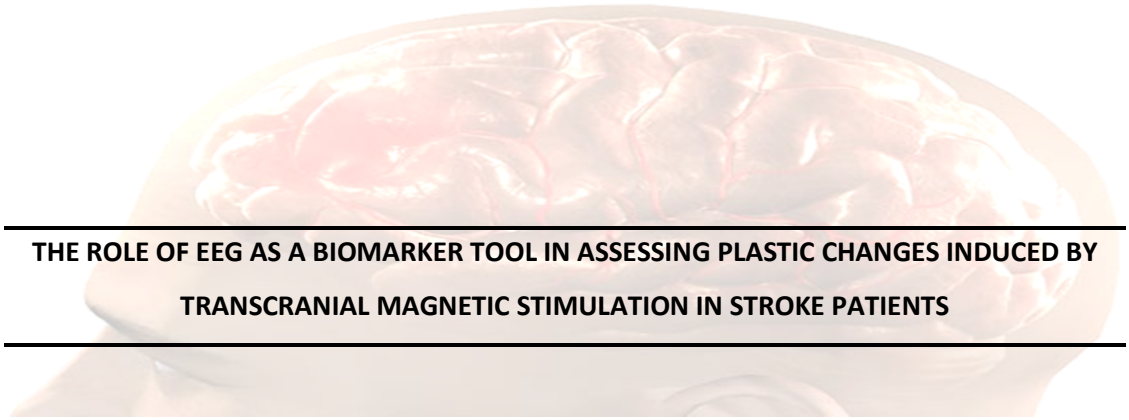
The patient think that was stimulated with ☐ Sham or ☐ Active

The patient was: ☐ Confident with his answer

☐ More or less confident with his answer

☐ Not confident with his answer

APPENDIX IV – Clinical report form for controls



THE ROLE OF EEG AS A BIOMARKER TOOL IN ASSESSING PLASTIC CHANGES INDUCED BY TRANSCRANIAL MAGNETIC STIMULATION IN STROKE PATIENTS

Admission date: __/__/____

Name:_____ Subject ID:____

Education level:_____ Job:_____

Address: _____

Phone number: _____

Edinburgh Handedness Inventory: _____

Structural MRI results: _____

EEG observations: _____

_____

TMS First time doing TMS? ☐ Yes ☐No

_____ HEMISPHERE

Muscle _____

Before cTBS MT _____ **Before cTBS** rMT _____

Active MT _____

_____ HEMISPHERE

Mean of MEP test/mean of MEP conditioning

Before		**After**	
MT	_____	MT	_____
rMT	_____	rMT	_____
SICI (1/3/5 ms)	___/___/___	SICI	___/___/___
ICF (10/15/20 ms)	___/___/___	ICF	___/___/___
LICI (50/100/150 ms)	___/___/___		

Side effects: _____

_____

APPENDIX V – <u>Sides Test Manual - Inventory Edinburgh</u>

Name:_____

Date: _____/_____/_____

	Put an X in the right column	Left	Both	Right
1	With which hand you usually write?			
2	With which hand you draw?			
3	Which hand you use to throw a ball and hit a basket?			
4	In which hand you use your tennis racket, squash, etc?			
5	In which hand you use your toothbrush?			
6	Which hand holds a knife when you cut things? (not using a fork)			
7	Which hand holds a hammer when you're pounding a nail?			
8	When you light a match, which hand holds the stick?			
9	In which hand you use an eraser on paper?			
10	What hand removes the top of the card when you are giving the cards? (Ex. When you are the player who gives the cards at the game, which hand you use to distribute the cards that will be placed on the table?)			
11	Which hand holds the line when you're tucking into a needle?			
12	In which hand you hold a 'kill-fly "(to kill a fly)?			
Subtotal				
Total				

33-36: Strongly right-handed
29-32: moderately right-handed
25-28: Weakly right-handed
24: Ambidextrous
20-23: Weakly left-handed
16-19: Moderately left-handed
12-15: Strongly left-handed

E = 1 point; A = 2 points; D = 3 points.

APPENDIX VI – Security Questionnaire for Transcranial Magnetic Stimulation

Name:_____ Date of birth:__ /__ /____	Investigator:_____ Date:__ /__ /____

To be completed by the participant:

1 - Do you have epilepsy or have you ever had a convulsion or a seizure?	□ Yes	□ No
2 - Have you ever had a fainting spell or syncope? If yes, please describe on which occasion(s)?_____	□ Yes	□ No
3 - Have you ever had a head trauma that was diagnosed as a concussion or was associated with loss of consciousness?	□ Yes	□ No
4 - Do you have any hearing problems or ringing in your ears?	□ Yes	□ No
5 - Do you have cochlear implants, ear canals or auditory implants?	□ Yes	□ No
6 - Are you pregnant or is there any chance that you might be?	□ Yes	□ No
7 - Do you have metal in the brain, skull or elsewhere in your body (e.g., splinters, fragments, clips, etc.)? If so, specify the type of metal._____	□ Yes	□ No
8 - Do you have an implanted neurostimulator (for vagus nerve stimulation, deep brain stimulation, epidural / subdural stimulation, ...)?	□ Yes	□ No
9 - Do you have a cardiac pacemaker or intracardiac lines?	□ Yes	□ No
10 – Do you have a medication infusion device or some intravenous infusion device drugs?	□ Yes	□ No
11 - Are you taking any medications, alcohol or drugs? (please list) _____	□ Yes	□ No
12 - Did you ever undergo TMS in the past? If so, were there any problems. _____	□ Yes	□ No
13 - Did you ever undergo MRI in the past? If so, were there any problems. _____	□ Yes	□ No

Participant signature:
_____, Coimbra __ /__/ ____

Investigator Signature:
_____, Coimbra __ /__/ ____

An affirmative answer to one or more questions of the numbered 1-11, is not absolute contraindication, but the risk / benefit should be calculated and should be given to the non-inclusion of the subject in the study.

APPENDIX VII – Security Questionnaire for MRI

Project nr.:
Exam nr.:
(to be filled by the service)

Surname:_____Name:_____Height:_____cm Weight:_____kg
Date of birth:____/____/____Phone:_____E-mail:_____
Address:_____Locality:_____
Postal code:_____ Municipality:_____
Contact name for urgency:_____Phone:_____
Doctor:_____Address:_____Phone:_____

1. Have you ever been submitted to any surgery and/or invasive procedure? Yes ☐ No ☐ (If affirmative, specify below)
   Type:_____Date:_____/_____/_____
   Type:_____Date:_____/_____/_____
2. Have you ever carried out any Magnetic Resonance Imaging? Yes ☐ No ☐ (If affirmative, specify below)
   Body area:_____Date:____/_____/_____Local:_____
   Body area:_____Date:____/_____/_____Local:_____
3. Have you worked as a machinist, with metal, or do you usually deal with metals? Yes ☐ No ☐
   Have you had any injury with metals in the eye? (p.e.: metallic pieces or foreign body) Yes ☐ No ☐
4. Are you (or can you) be pregnant or breastfeeding? Yes ☐ No ☐
5. Do you suffer from sickle-cell anemia or thalassemia? Yes ☐ No ☐
6. Do you have pacemaker or an implanted heart defibrillator? Yes ☐ No ☐

The Magnetic Resonance Imaging (MRI) uses a very high magnetic field, quickly modified magnetic field gradient and uses high radiofrequencies. Some metallic and electromagnetic objects can interfere with the exam and even be dangerous. Before you are allowed to enter, we must know if you have any metallic object in your body, electromagnetic equipment or if you fit in some of the circumstances described bellow. Please answer correctly.

Yes ☐ No ☐ Aneurysm clip or cerebral clip
Yes ☐ No ☐ Vascular clamp in the carotid artery
Yes ☐ No ☐ Neurostimulator
Yes ☐ No ☐ Insulin or infusion pump
Yes ☐ No ☐ Cochlear implant, ear canals or ear implant
Yes ☐ No ☐ Prostheses (eye/orbit, etc)
Yes ☐ No ☐ Implant placed by a strong magnet
Yes ☐ No ☐ Prostheses of cardiac valves
Yes ☐ No ☐ Artificial limb or joint
Yes ☐ No ☐ Other implants in the body or head
Yes ☐ No ☐ Electrodes (body, head or brain)
Yes ☐ No ☐ Intravascular stents, filters or other similar devices
Yes ☐ No ☐ Shunt (intraventricular or cerebral)
Yes ☐ No ☐ Catheters or vascular access port
Yes ☐ No ☐ Intrauterine device or diaphragm
Yes ☐ No ☐ Adhesives or therapeutic dressings (p.e.: nicotine, birth control, pain, etc)
Yes ☐ No ☐ Metallic shrapnel or bullets
Yes ☐ No ☐ Tattoos
Yes ☐ No ☐ Eye shadow **(remove before enter)**
Yes ☐ No ☐ Piercings **(remove before enter)**
Yes ☐ No ☐ Metallic fragments (p.e.: eye, skull, body)
Yes ☐ No ☐ Aortic clip
Yes ☐ No ☐ Metallic implants or wire mesh
Yes ☐ No ☐ Surgery staples or sutures
Yes ☐ No ☐ Harrington bars (column)
Yes ☐ No ☐ Fastener, screw or plate in the bone/joint
Yes ☐ No ☐ Wig **(remove before enter)**
Yes ☐ No ☐ Fake hair
Yes ☐ No ☐ Hearing aid **(remove before enter)**
Yes ☐ No ☐ Dentures **(remove before enter)**
Yes ☐ No ☐ Dental implants
Yes ☐ No ☐ Asthma or respiratory diseases
Yes ☐ No ☐ Dizziness, epilepsy or motor incoordination
Yes ☐ No ☐ Hospitalization by mental or neurological problems
Yes ☐ No ☐ Head trauma
Yes ☐ No ☐ Migraine or migratory headache
Yes ☐ No ☐ Panic attacks
Yes ☐ No ☐ Infarct or stroke
Yes ☐ No ☐ Health problems when laying on your back
Yes ☐ No ☐ Problems completing previous MRI exam
Yes ☐ No ☐ Claustrophobia

Please remove every metallic objects before entering the MRI room including: keys, pins for the hair, earrings, watches, necklaces, bracelets, pens, belts, metallic buttons, metallic props (p.e.: brooches, pins, etc), clips, coins,

pocket knife and clothes with metal. It is required ear protection during the exam of MRI.

I confirm that the above information is correct according to my best knowledge. I have read and understood every questions and terms referred in this form. It was given me the opportunity to ask every question that I found necessary and my doubts regarding this form were all clarified.

Signature: _____Date:_____/_____/_____
Conferred by:_____ Date:_____/_____/_____

APPENDIX VIII – Acquisition Lab

Figure A1. Acquisition lab

APPENDIX IX – Wolf Motor Function Test

Patient ID: _____

Task	**BEFORE** cTBS Time (sec)	**BEFORE** cTBS FAS (0-5)	**AFTER** cTBS Time (sec)	**AFTER** cTBS FAS (0-5)	**3 Months Follow-up** Time (sec)	**3 Months Follow-up** Time (sec)
	Affected Member	Affected Member	Affected Member	Affected Member	Affected Member	Affected Member
Forearm to table (side)						
Forearm to box (side)						
Extend elbow (side)						
Extend elbow (weight)						
Hand to table (front)						
Hand to box (front)						
Reach and retrieve						
Lift can						
Lift pencil						
Lift paper clip						
Stack checkers						
Flip cards						
Turn key in lock						
Fold towel						
Lift basket						
TOTAL						

Table 8. Data Entry Form- Wolf Motor Function Test

APPENDIX X – Wolf Motor Function Test

(A)

(B)

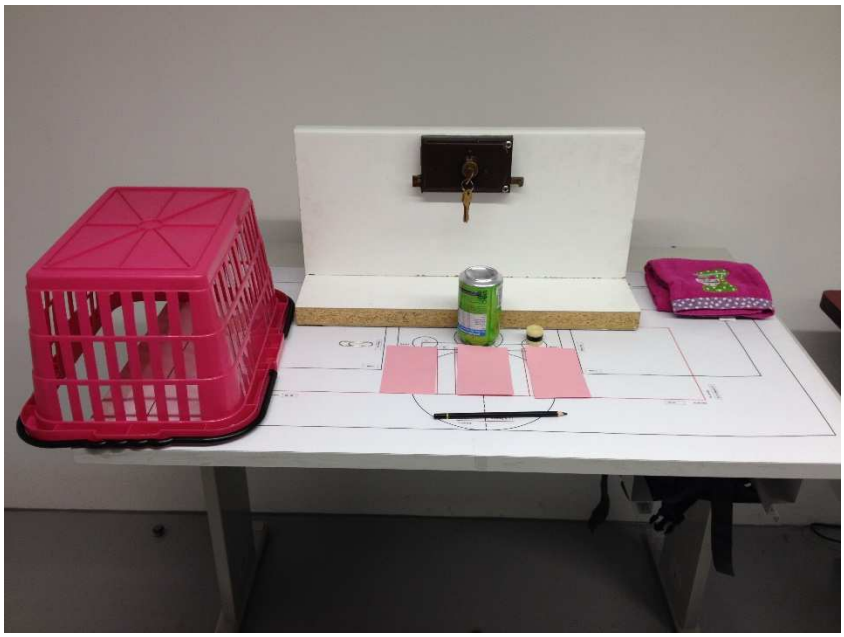


Figure A2. (A) Standardized test item template taped to the desk; (B) Equipment required to perform the WMFT: individual wrist weights, pencil with 6 flat sides, paper clip, checkers, three note cards, standardized lock and key board at 45 degree angle, standardized face towel, standardized basket and beverage can.

APPENDIX XI – EEG and EMG setup

(A)

(B)

(C)

(D)

Figure A3. (A) Equipment required to perform EEG and EMG: gloves, swabs, alcohol, Nuprep, two 25 ml syringes, tape, EEG cap which connects to image B, EEG cap is filled with Electro-Gel, three EMG electrodes which connects to image D and EMG electrodes are filled with Ten20 conductive paste; (B) Head box which connects to EEG cap and image C; (C) NeuroScan amplifier which connects to the computer; (D) Biopac system used for EMG which connects to the computer.

APPENDIX XII – Neuronavigation setup

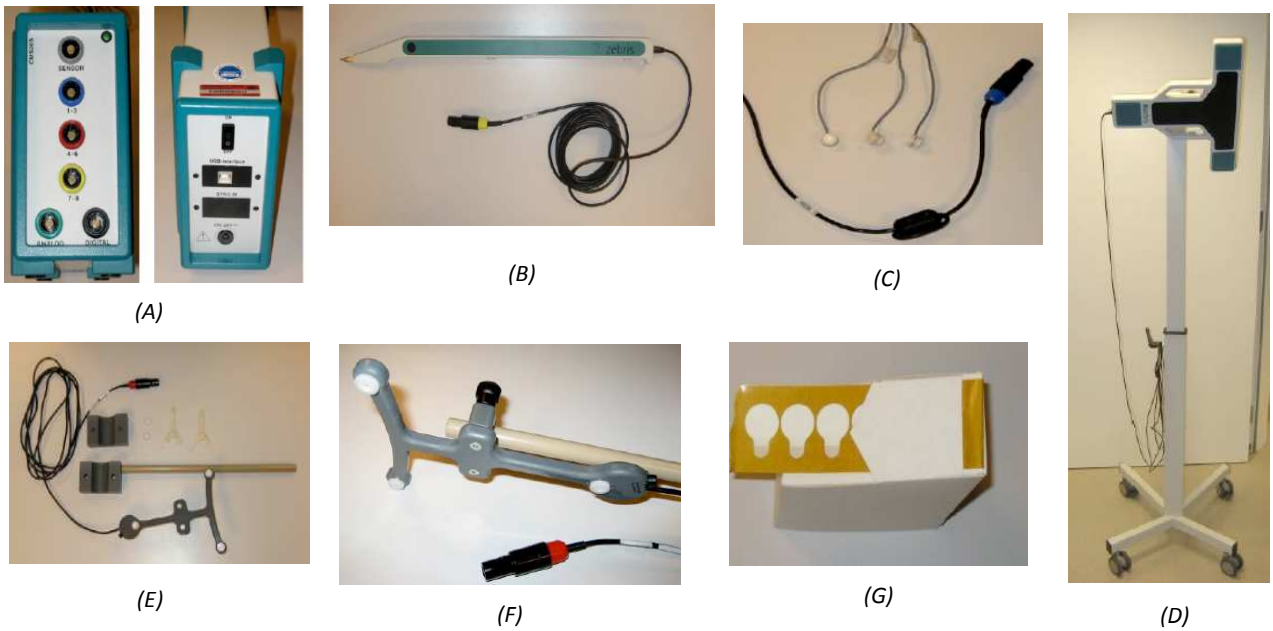
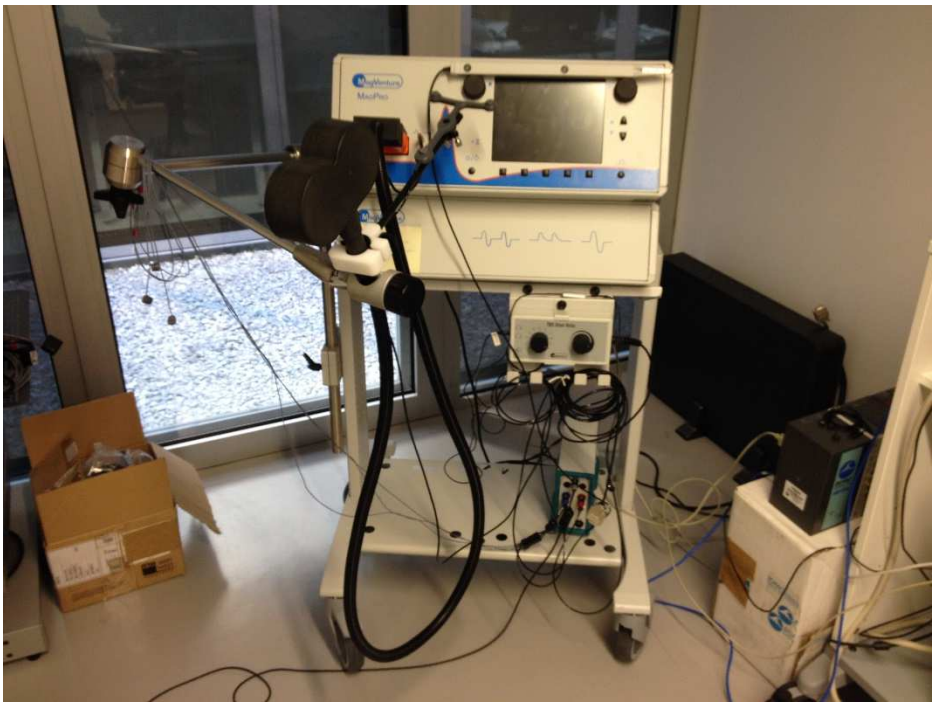


Figure A4. (A) Main Unit; (B) Pointer (digitizer pen); (C) Three ultrasound marker with adapter; (D) MAXX-2 with Y-shape design; (E)TMS coiler holder; (F) Triple Marker; (G) Adhesive Stickers (C. Goebel, et al. 2012. TMS Neuronavigation for CMS20 Measuring System)

APPENDIX XIII – TMS setup

(A)



(B)

(C)

Figure A5. (A) Transcranial Magnetic Stimulation machine; (B) Earplugs; (C) Earphones.

APPENDIX XIV – Schematic representation of the experimental procedure in stroke patients

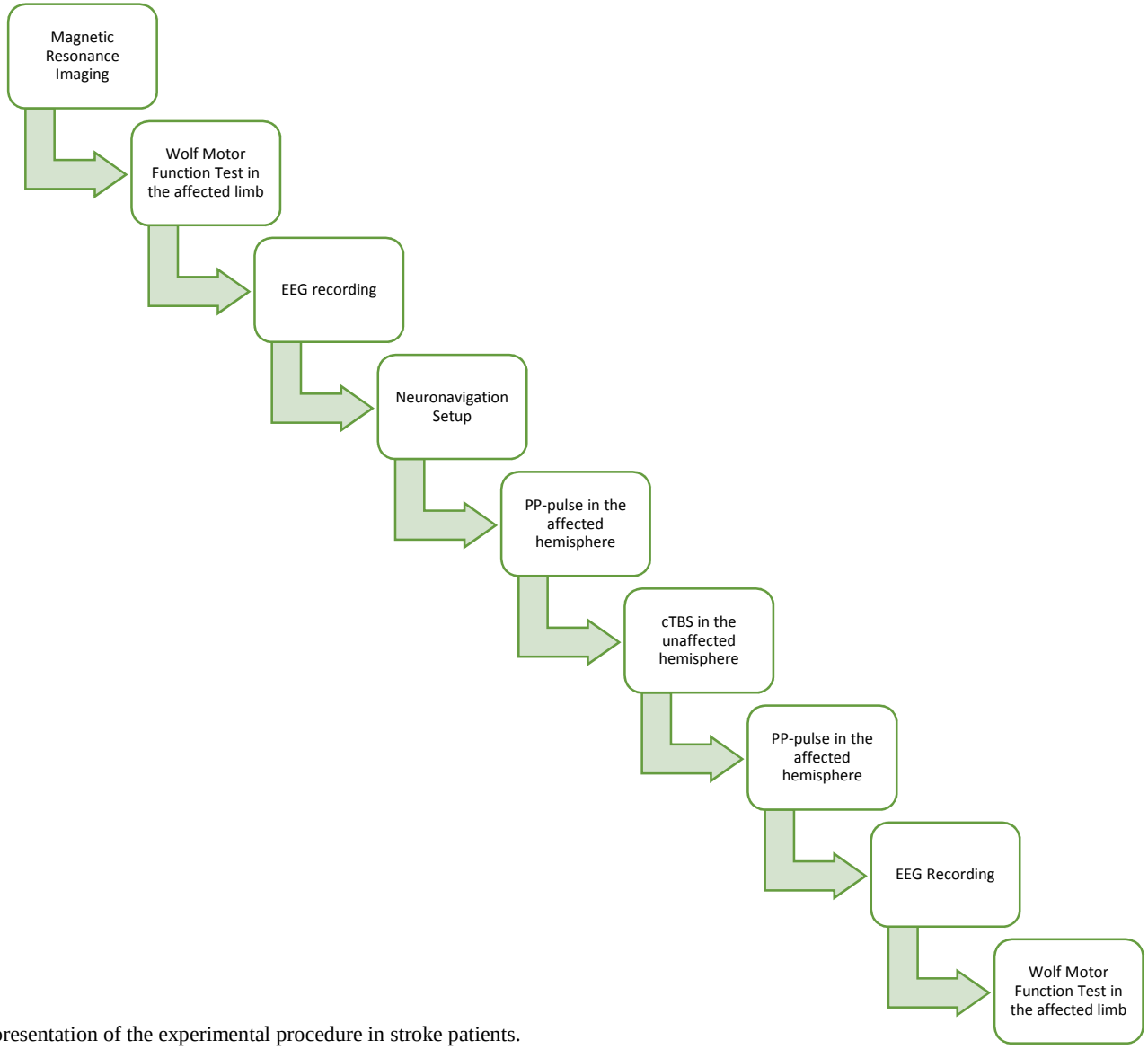


Figure A6. Schematic representation of the experimental procedure in stroke patients.

APPENDIX XV – Schematic representation of the experimental procedure in control subjects

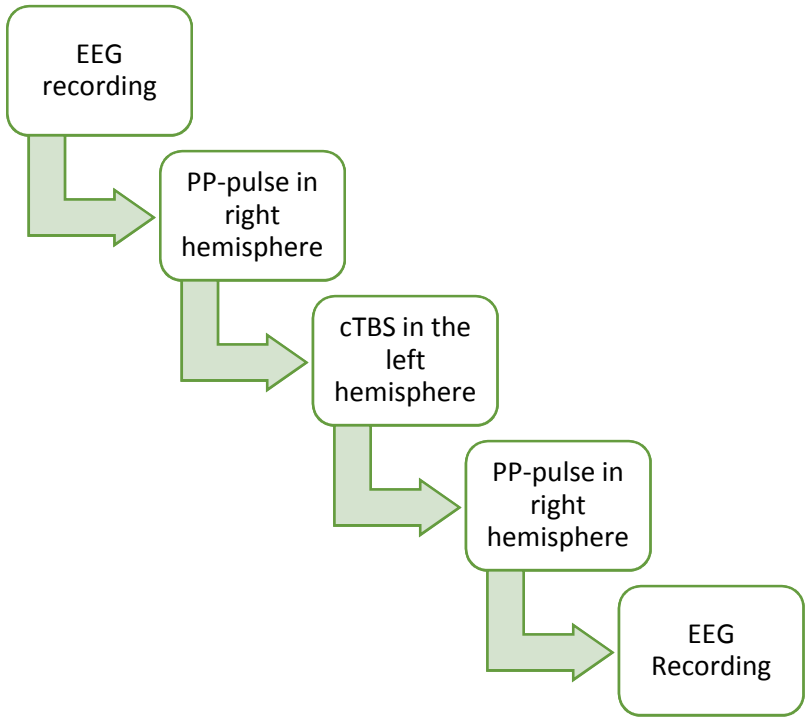


Figure A7. Schematic representation of the experimental procedure control subjects stimulated on the left hemisphere

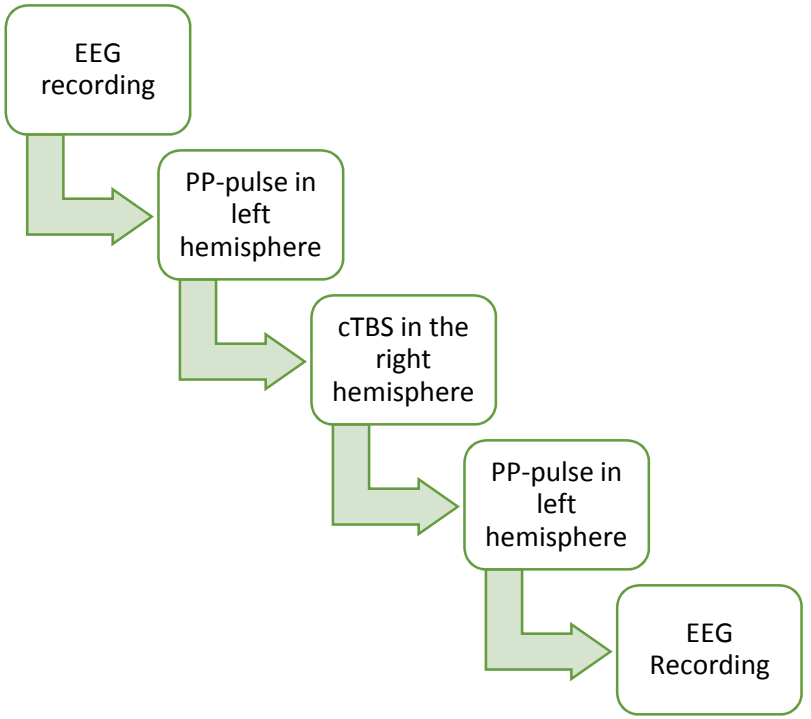


Figure A8. Schematic representation of the experimental procedure control subjects stimulated on the right hemisphere