EXPLORATORY RESEARCH ON THE EFFECTS OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION (rTMS) ON MUSCULOSKELETAL BIOMARKERS ON STROKE PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

by

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LIST OF SYMBOLS AND ABBREVIATIONS

ADP	Adenosine diphosphate
Ag/AgCl	Silver/silver chloride
AH	Affected hemisphere
AMPARs	α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors
ANS	Autonomic nervous system
ATP	Adenosine triphosphate
BDI	Beck Depression Inventory
BDNF-TrkB	Brain-derived neurotrophic factor- tropomycin receptor kinase B
CAT	Computerized Axial Tomography
CGI	Clinical Global Impression
CIMT	Constraint-induced movement therapy
CK	Creatine Kinase
CMIA	Chemiluminescent microparticle immunoassay
CNS	Central nervous system
cTBS	Continuous theta burst stimulation

DALYs	Disability-adjusted-life years
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyography
ER	Endoplasmic reticulum
FDI	First dorsal interosseous
fMRI	Functional magnetic resonance imaging
G-6-P	Glucose-6-phosphate
G-6-PDH	Glucose-6-phosphate dehydrogenase
GABA	Gamma-aminobutyric acid
GBD	Global Burden of Disease
HDL	high-density lipoprotein
hNSCs	Human neural stem cells
HUSM	Hospital Universiti Sains Malaysia
Hz	Hertz
iNOS	Inducible nitric oxide synthase
ISI	Inter-stimulus interval

iTBS	Intermittent theta burst stimulation
LTD	Long term depression
LTP	Long term potentiation
M1	Primary motor cortex
MADRS	Montgomery–Åsberg Depression Rating Scale
MDD	Major depressive disorder
MEP	Motor evoked potential
mm	Millimeter
mmHg	Millimeters of mercury
mtPTP	Mitochondrial permeability transition pore
NAC	N-acetyl-L-cysteine
NADP+	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NF-kB	Nuclear factor kappa B
ng/mL	Nanograms per milliliter
NMDARs	N-methyl-D-aspartate receptors
NO	Nitric oxide

NOS	Nitric oxide synthase
NRF2	Nuclear factor erythroid 2–related factor 2
NSCs	Neural stem cells
ONOO-	peroxynitrite
PAS	Paired associative stimulation
PCr	Phosphocreatine
PET	Positron emission tomography
rMT	Resting motor threshold
RPM	Revolutions per minute
rTMS	Repetitive Transcranial Magnetic Stimulation
SERCA	Sarcoplasmic/ER calcium ATPase
SPECT	Single-photon emission computed tomography
SSRI	Selective serotonin reuptake inhibitors
SVZ	Subventricular zone
TBS	Theta burst stimulation
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation

TNF- α	Tumor necrosis factor- α
TnT	Troponin T
TOAST	Trial of Org 10172 in Acute Stroke Treatment
U/L	Units per liter
UH	Unaffected hemisphere
USM	Universiti Sains Malaysia

PENYELIDIKAN AWAL KESAN STIMULASI

MAGNETIK TRANSKRANIAL BERULANG (rTMS) TERHADAP

BIOMARKER MUSCULOSKELETAL PADA PESAKIT ANGIN AHMAR

DI HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRAK

Penyebab utama kecacatan jangka panjang disebabkan oleh angin ahmar ialah fungsi motor yang terjejas. “Neuroplasticity” ialah suatu perubahan yang berlaku di bahagian korteks dan merupakan proses penting untuk pemulihan fungsi motor setelah serangan angin ahmar. Rangsangan magnetik transcranial berulang (rTMS) merupakan sejenis rangsangan otak tidak invasif, yang diguna untuk menggalakkan proses “neuroplasticity” dengan mengubah rangsangan di bahagian kortikal otak. Kami menguji kesan rTMS apabila rangsangan magnetik diberi ke korteks motor utama (M1) di pada bahagian otak yang tercedera. Kajian terdahulu telah melaporkan peningkatan tahap serum Creatine Kinase (CK) dan Troponin T di kalangan individu yang mengalami angin ahmar. Enzim serum ini digunakan secara meluas sebagai biomarker untuk mengenalpasti fungsi otot. Tujuan kajian ini adalah untuk menilai perubahan dalam tahap CK dan Troponin T sebagai tindak balas kepada 10 Hz rTMS pada individu yang mengalami serangan angin ahmar (jenis iskemik dan hemoragik).

Seramai lapan pesakit angin ahmar yang memenuhi kriteria inklusi dan pengecualian telah dibenarkan untuk menyertai kajian ini. Mereka menjalani 10 sesi

protokol “fascilitatory” rTMS pada bahagian otak serangan angin ahmar selama dua minggu. Sampel darah pesakit telah diperoleh sebelum dan selepas intervensi rTMS untuk menganalisis tahap serum CK dan Troponin T. Analisis statistik menunjukkan bahawa tiada perubahan signifikan dalam nilai median CK dan Troponin T sebelum dan selepas rTMS. Analisis deskriptif menunjukkan bahawa nilai median CK dan Troponin T sebelum sesi rTMS bermula adalah lebih tinggi daripada nilai selepas rTMS dalam sampel ini. Tambahan lagi, pesakit strok dalam kategori umur yang lebih tua didapati mempunyai tahap CK dan Troponin T yang lebih tinggi berbanding pesakit yang lebih muda. Konklusinya, terdapat perbezaan dalam aktiviti CK dan Troponin T dalam setiap pesakit angin ahmar, dan ia disebabkan oleh beberapa pembolehubah dan saiz sampel yang agak kecil. Oleh itu, kajian masa hadapan adalah dicadangkan untuk menganalisis kesan faktor-faktor seperti status aktiviti fizikal, sejarah perubatan pesakit dan jenis ubat-ubatan ke atas tahap serum biomarker tersebut, untuk digunakan sebagai penilai integriti sel-sel otot selepas rTMS.

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ABSTRACT

The leading cause of long-term disability caused by stroke is motor impairment. Neuroplasticity-induced cortical reorganization is a crucial process mediating motor recovery following stroke. Repetitive transcranial magnetic stimulation (rTMS) is a form of non-invasive brain stimulation approach that promotes neuroplasticity by modulating cortical excitability. We tested the effects of rTMS, when delivered over the ipsilesional hemisphere at the site of primary motor cortex (M1). Previous studies have reported elevated levels of serum Creatine Kinase (CK) and Troponin T among individuals with stroke. These serum enzymes are widely used as biomarkers for functional status of skeletal muscles. The aim of the present study is to assess the changes in the CK and Troponin T levels in response to 10 Hz rTMS in individuals with ischemic and hemorrhagic stroke.

Eight stroke patients were screened according to inclusion and exclusion criteria. Subsequently, they were subjected to 10 sessions of fascilitatory protocol of rTMS on the ipsilesional hemisphere for a period of two weeks. The blood samples of the patients were obtained pre- and post-intervention in order to analyze the levels of CK and Troponin T. Statistical analysis revealed that there is no statistically significant difference in the median

values of CK and Troponin T pre- and post-rTMS. Descriptive analysis indicated that the median values of CK and Troponin T at the baseline are higher than post-rTMS in this sample. In addition, stroke patients in the older age group were found to have higher levels of CK and Troponin T as compared to the younger patients. The considerable interindividual variability in CK and Troponin T activities among stroke patients can be ascribed to several confounding variables and relatively small sample size. Therefore, it can be suggested that future studies may include factors such as physical activity status, medical history of patients and types of medications in the analysis in order to assess the serum levels of aforementioned biomarkers, as a reliable indicator of muscle cells integrity following rTMS.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Stroke is a neurological condition that occurs in the central nervous system and causes wide range of cognitive and physical deficits. This neurological disorder is also known as cerebrovascular accident among medical community. It occurs when the blood supply to a region of the brain is obstructed, potentially causing the death of neural tissues due to insufficient supply of glucose and oxygen. Stroke can be classified into two broad categories, namely ischemic and hemorrhagic stroke. Most of the strokes happen due to occlusion of the blood supply by a clot, known as ischemic stroke. Hemorrhagic stroke, on the other hand, occurs when a blood vessel within the brain ruptures, causing bleeding in the brain (Benjamin et al., 2018).

The Global Burden of Disease (GBD) 2013 study showed that stroke is a major cause of death and disability in many countries. In 2013 alone, it was reported that there were approximately 6.5 million deaths due to stroke, 25.7 million stroke survivors, 113 million disability-adjusted-life years (DALYs) lost as well as 10.3 million new cases of stroke worldwide (Feigin et al., 2015). The 2018 statistics in Malaysia has indicated

cerebrovascular accident as the third leading cause of deaths in the country, preceded by ischemic heart diseases and pneumonia (Mahidin, 2018).

Approximately 90% of the causes of stroke are due modifiable factors, and thus, most strokes could be precluded by controlling these factors, which are predominantly metabolic and behavioral in nature. The six most crucial causes are hypertension, diabetes mellitus, hypercholesterolemia, obesity, smoking and insufficient physical activity (Hsieh & Chiou, 2014). High blood pressure is accounted as the most common risk factor affecting populations worldwide, followed by diabetes mellitus and smoking behavior. A review on stroke epidemiology among Asian countries reported that Malaysia has a high prevalence of physical inactivity (Venketasubramanian, Yoon, Pandian, & Navarro, 2017). Moreover, some factors that are uncontrollable also increase the risk of stroke, such as age and gender.

One of the most commonly altered functions of the brain following stroke is the motor ability. An impaired motor function occurs as a result of the damage to a brain region associated with motor control, which is comprised of both cortical and subcortical structures of the brain. Occlusion of cerebral blood flow in brain regions that are responsible for the generation and control of voluntary movement, such as primary motor cortex (M1), premotor area, parietal cortex, cerebellum and basal ganglia, may result in motor deficits. M1 is a primary source of descending neural projections from the cerebral cortex to the motor neurons in the spinal cord and the cranial nerve nuclei. M1 is located in the precentral gyrus (Brodmann's area 4) (Figure 1.1). One of the crucial characteristics of M1 is that the neurons in this cerebral region can readily be stimulated to produce motor

effects in response to electrical stimulus (Kandel, Schwartz, & Jessell, 1991; Purves et al., 2004).

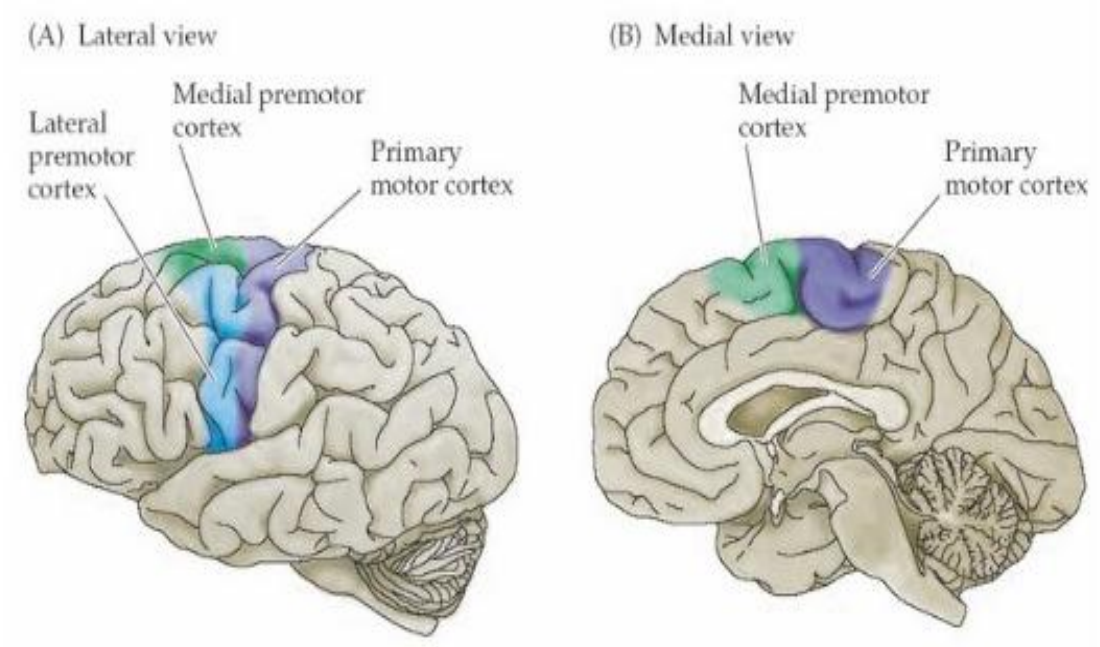

Figure 1.1: Lateral (A) and medial (B) views of the primary motor cortex (M1) and premotor cortex.

Treatment of cerebrovascular accident can be classified into two major categories, which are neuropharmacological and neurorehabilitation approaches. Pharmacological interventions often targeted at reducing the amount of brain injury due to ischemia, whereas rehabilitative strategies are implemented to involve physical, occupational and speech therapy that rely on neuroplasticity to promote recovery of motor functions. Any therapeutic modality used for neurological rehabilitation is based on assumptions about the functioning of central nervous system. Novel therapeutic strategies for stroke rehabilitation

have started to surface in the last few years. Some of the main reasons driving these novel techniques are attributable to enhanced understanding of motor control and motor learning.

Repetitve transcranial magnetic stimulation (rTMS) is a non-invasive brain stimulation approach that has the potential to modulate neuroplasticity in order to elicit motor learning, following stroke (F. C. Hummel & Cohen, 2005). The interhemispheric imbalance model suggests that disinhibition of contralesional motor regions correlated with increased inhibition of ipsilesional motor regions (Duque et al., 2005). Due to these underpinnings, the goals of rTMS protocols can be either to enhance the cortical excitability in ipsilesional cortical regions using fascilitatory protocol of rTMS or to depress the cortical excitability in contralesional cortical regions using inhibitory protocol of rTMS (Yozbatiran et al., 2009).

Stroke results in long-term dysfunctions due to complex pathomechanisms, and one of them is neuroinflammation (R. Liu et al., 2017). Enormous research have been directed to identify potential neuronal anti-inflammation agents in controlling the effects of cerebral ischemia to the central nervous system (CNS). However, the impact of neuroinflammation on the skeletal muscle and peripheral nerve is still poorly understood.

Skeletal muscle tissue in stroke patients may be disrupted following the over usage or the underusage of skeletal muscles. In stroke patients, hemiparesis leads to underusage

of skeletal muscles in the affected limbs. This condition is further aggravated by disruptions in carbohydrate metabolism that leads to hypermetabolic state, resulting in decreased ATP availability and insufficiency of Na-K ATPase. This will lead to increased membrane permeability in skeletal muscle and thus indirectly cause muscle damage. This cellular event can be indicated by measuring the levels of serum enzymes, namely Creatine Kinase (CK) and Troponin T.

Previous studies have only focused on biomarkers of restorative therapies in CNS following stroke, including several neuroimaging indicators (Burke & Cramer, 2013). In contrast, current research evaluates the effects of rTMS on motor recovery, by measuring serum musculoskeletal biomarkers of stroke patients before and after intervention. These sera skeletal muscle enzymes indicate the functional status of skeletal muscles. As permeability of skeletal muscle membrane is disrupted in stroke, the muscle damage can be indirectly assessed by these relatively cheap, readily available biomarkers.

It has been stipulated that neuroplasticity induced by rTMS intervention may improve motor performance among patients, which in turn enhances the integrity of skeletal muscle cells and reduces the levels of these biomarkers. Therefore, this study attempts to examine the changes in levels of creatine kinase (CK) and Troponin T pre- and post-rTMS treatment. If the findings obtained in this study indicate changes on the skeletal muscle level, a neurorehabilitative guideline can be established in hospital USM to enhance motor recovery among stroke patients.

1.2 Problem Statement

Although constraint-induced movement therapy (CIMT) is notable in improving motor recovery to a certain extent among stroke patients, a full recovery is rarely achieved and further studies are required to elucidate its molecular mechanisms (Corbett, Sirtori, Castellini, Moja, & Gatti, 2015). The major limitation of traditional methods of neurorehabilitation, which are based on direct stimulation of muscles and peripheral nerves in order to indirectly influence the central nervous system (CNS), is that they have a moderate effect on neuroplasticity (Mohammadi, 2016). The review by Cochrane has selected 15 studies with 455 stroke patients and could not conclude that there was a strong evidence of efficacy of transcranial direct current stimulation (tDCS) (Elsner, Kugler, Pohl, & Mehrholz, 2013).

As an alternative, it has been postulated that direct stimulation of the CNS by changing the electromagnetic field may contribute further alterations in the intracortical connections which are related to motor recovery, with the use of rTMS. Repetitive transcranial magnetic stimulation (rTMS) is a neuromodulatory tool that is entirely non-invasive, utilized to induce neuroplasticity in order to produce therapeutic effects on motor impairment among stroke patients. Since the protocols of rTMS varied, it requires extensive pre-clinical and clinical trials to determine which specific parameter is best used as a neurorehabilitative approach.

The aim of this research is to assess the effects of 10 Hz rTMS over ipsilesional primary motor cortex (M1) that is conducted for 10 consecutive days on musculoskeletal

biomarkers, namely creatine kinase (CK) and troponin T. The outcome measure is the levels of musculoskeletal serum biomarkers, namely Creatine Kinase and Troponin T. The underusage of motor movement among stroke patients causes the levels of these biomarkers to be increased in the serum plasma due to skeletal muscle injury. Therefore, rTMS is postulated to improve the motor abilities among stroke patients, which in turn causes the drop in the levels of aforementioned biomarkers.

1.3 Rationale

Current research is intended to examine the effects of high frequency repetitive Transcranial Magnetic Stimulation (rTMS) on changes in musculoskeletal biomarkers, which are Creatine Kinase and Troponin T. Fascilitatory protocol of rTMS involving 10 Hz stimulation will be applied to the injured brain hemisphere. The design of this study does not require pain relieving method during the treatment and hospital stay might be needed during the procedure. If promising results are obtained, this study will become a guide to our health professionals to conduct an alternative neurorehabilitative approach using non-invasive rTMS to promote motor recovery among stroke patients. As the musculoskeletal biomarkers have shown notable results in stroke recovery prognosis, rTMS can be utilized as a nonpharmacological strategy for rehabilitation. This study can also direct future researchers to conduct extensive researches on therapeutic effects of non-invasive neurostimulation approach in promoting the well-being of stroke patients, as well as to improve motor abilities in patients of other neurological diseases.

1.4 Research Questions

There are two questions intended to be answered by conducting this research, which are:

1. Is there any statistical significant difference in the levels of serum creatine kinase (CK) between pre- and post- rTMS intervention?

2. Is there any statistical significant difference in the levels of serum Troponin T between pre- and post- rTMS intervention?

1.5 Research Objectives

1.5.1 General objective

To evaluate the changes in the levels musculoskeletal biomarkers pre- and post- rTMS.

1.5.2 Specific objectives:

1. To determine the changes in the levels of serum Creatine Kinase before and after high frequency repetitive transcranial magnetic stimulation (rTMS) is administered on ipsilesional hemisphere of stroke patients.

2. To determine the changes in the levels of serum Troponin T before and after high frequency repetitive transcranial magnetic stimulation (rTMS) is administered on ipsilesional hemisphere of stroke patients.

CHAPTER 2

LITERATURE REVIEW

2.1 Etiologic classification of stroke

Stroke was first described more than 2400 years ago in Hippocratic Writings using the term “apoplexy” (Clarke, 1963), which means the paralysis of part of the body due to an apoplectic attack or possibly other causes. Scholars in 17th century had reached out for new horizons, leaving the doctrines of Hippocrates and Galen behind them. During this time before the discovery of arterial circle by Thomas Willis, a Swiss physician, Jacob Wepfer, indicated that apoplexy happens due to cerebral hemorrhage (J. Pearce, 1997). He was the first researcher who pointed out that apoplexy could result from either the occlusion of one of the main arteries to the brain (ischemic stroke) or bleeding in the brain (hemorrhagic stroke) (Fields & Lemak, 1989).

Strokes can be categorized into two distinctive groups, which are ischemic and hemorrhagic stroke. These two main classifications allow further classification into subtypes, as represented in Figure 2.1. By applying clinical evaluations to neurological modalities such as Computerized Axial Tomography (CAT) scan, standard 24-hours

electrocardiography, echocardiography and ultrasound of intra and extracranial arteries, the most probable etiology is determined by the physician.

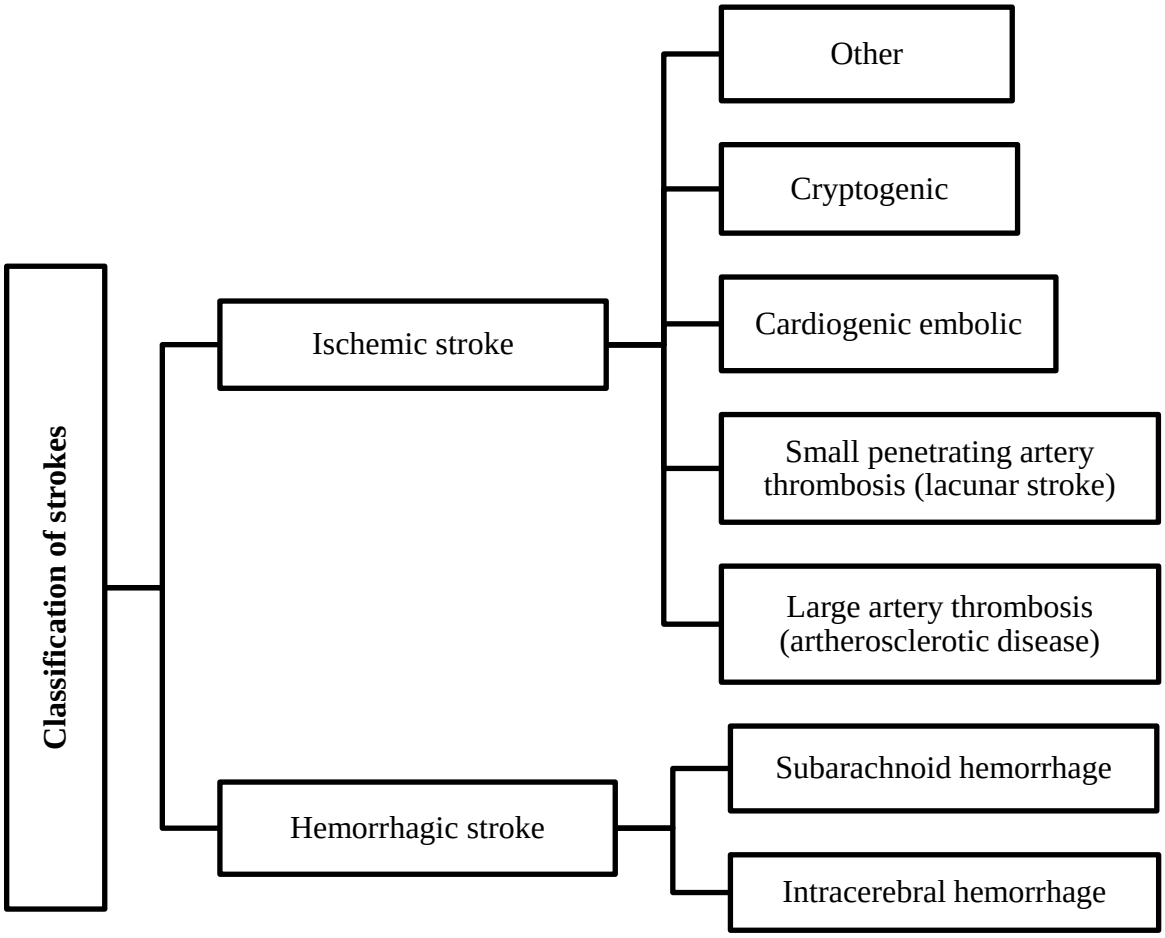

Figure 2.1: Classification of Strokes. Source: (Adams et al., 1993)

According to the TOAST classification system, ischemic stroke can further be categorized into five subtypes as due to large-vessel atherosclerosis, cardioembolic origin, small vessel disease, other determined causes, and stroke of undetermined causes (Adams et al., 1993).

On the other hand, hemorrhagic stroke is less common than ischemic stroke, and can be subdivided into two types, which are subarachnoid and intracerebral hemorrhage. In intracerebral hemorrhage, bleeding occurs directly into the functional tissues of the brain that are comprised of neurons and glial cells (Domingues, Rossi, & Cordonnier, 2014). One of the causes is due to chronic intracranial hypertension, small intracerebral arteries thought to be leaked and cause apoplexy. Besides the affected brain region, the surrounding sites can also be damaged due to the pressure inflicted by the mass effect of the hematoma, causing more intense intracranial pressure. Another type of hemorrhagic stroke is subarachnoid hemorrhage, which is an uncommon stroke that is potentially life threatening (van Gijn & Rinkel, 2001). Similar to intracranial hemorrhage, subarachnoid hemorrhage also aggravates the intracranial pressure with combination of several other signs such as aggregation of microvascular platelets, acute vasoconstriction and loss of microvascular perfusion. Eventually, all these signs lead to significant reduction in cerebral blood flow and cause cerebral ischemia.

2.2 Pathophysiology of stroke

The reduction or complete occlusion of cerebral blood flow that occurs during a cerebrovascular accident often leads to a complex pathophysiological response due to neuronal injury, as depicted in Figure 2.2 (Hossmann, 2006). A series of events including excitotoxicity mechanisms, inflammatory pathways, protein misfolding, the release of free radicals and mitochondrial response will take place and eventually lead to the death of neural cells. Subsequently, these cascades of cellular and molecular pathways essentially promote post-stroke recovery.

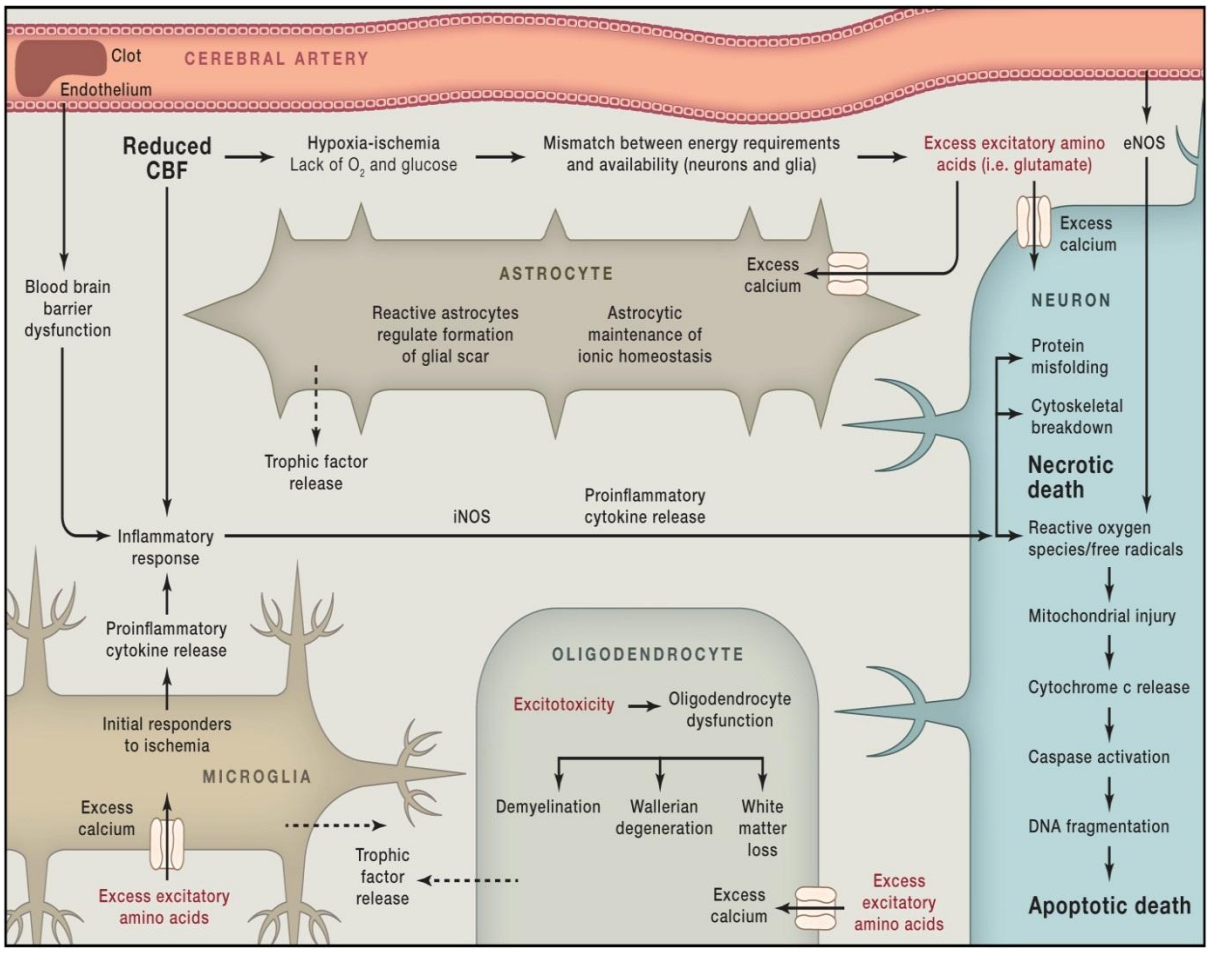

Figure 2.2: Pathophysiology of Stroke. Source: (Hossmann, 2006).

2.2.1 Excitotoxicity mechanisms

The formation of thrombus or embolus in the cerebral artery leads to inadequate supply of oxygen and glucose to the brain tissues due to restricted blood flow to the brain. This condition is known as ischemic hypoxia. As a result, neuronal cells fail to sustain normal ionic gradients. Depolarization of these neurons leads to excessive glutamate release that eventually leads to intracellular influx of calcium, causing cell death mechanisms such as necrosis, autophagocytosis and apoptosis (Lipton, 1999). This course

of actions has been termed excitotoxicity and is largely intervened by glutamatergic synaptic transmission involving N-methyl-D-aspartate receptors (NMDARs), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors (AMPARs) and kainate receptors (Dirnagl, Iadecola, & Moskowitz, 1999; Moskowitz, Lo, & Iadecola, 2010). Excessive intracellular calcium further leads to a cascade of events, such as mitochondrial impairment and activation of oxidative molecules, phospholipases and proteases. These mechanisms account for neural cell damage or death (Szydlowska & Tymianski, 2010).

2.2.2 Inflammatory pathways

Intracranial artherosclerotic disease is a common cause of stroke that is associated with a high risk of recurrent stroke (Banerjee & Chimowitz, 2017). Inflammatory pathways imply significant roles in the development of atherosclerotic plaque and initiated when damage to endothelium occurs (Gimbrone & García-Cardeña, 2013).

Inflammatory pathways comprised of a complex interaction of immune cells and inflammatory factors that lead to blood-brain barrier breakdown, reorganization of brain tissue post-stroke and also provide neuroprotection from the enzymes and free radicals that were produced due to excitotoxic environment post-stroke (Iadecola & Anrather, 2011). Immune cells release proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-1β, as well as oxidative molecules in the neural tissues following stroke in order to propagate the immune response (J. Huang, Upadhyay, & Tamargo, 2006). As response to cytokines, inducible nitric oxide synthase (iNOS) is also released and has damaging effects in stroke (Moro, Cardenas, Hurtado, Leza, & Lizasoain, 2004). Earlier

research conducted by Arac et. al (2011) has proven that impeding acute inflammatory response following stroke leads to less damage and enhance neurological outcome in rodent models.

Inflammatory mechanism has both positive and negative consequences on the surviving brain tissues. The timing and levels of interaction between immune cells and inflammatory factors are accounted for balance of damage after stroke and the recovery process (Peruzzotti-Jametti et al., 2014). While immune cells produce cytokines and radicals that aggravate inflammatory response, they also help in eliminating post-stroke debris and damaged tissue in order to promote recovery of retaining tissues.

2.2.3 Protein misfolding

Endoplasmic reticulum (ER) is an organelle that holds large stores of intracellular calcium and is responsible for protein synthesis and responds to protein misfolding (Zhang et al., 2015). Stroke or cerebral ischemia leads to ER stress and has effect on these cellular and molecular mechanisms (Roussel et al., 2013). Excitotoxic environment following stroke causes dysfunction of sarcoplasmic/ER calcium ATPase (SERCA) pump due to lack of energy, which in turn aggravates neural cell death (Szydlowska & Tymianski, 2010).

2.2.4 Release of free radicals

Free radicals are oxygen-containing molecules with an uneven number of electrons that are released due to ischemic condition of the brain tissues. They easily react with other

biochemical molecules within the cellular environment. Previous research has shown that the influx of calcium triggers NO synthase (NOS) to secrete nitric oxide (NO) that leads to cell damage through the productions of peroxynitrite (ONOO-) and oxygen free radicals (Iadecola, 1997). The mitochondrial impairment that happens due to inadequate supply of blood to the brain also adds on oxidative damage (Kalogeris, Bao, & Korthuis, 2014). In addition to initial toxicity, free radicals also impede recovery, which makes them a crucial therapeutic target for stroke intervention (Miyamoto et al., 2013).

2.2.5 Mitochondrial response

When the supply of oxygen to the brain is interrupted during cerebrovascular event, the neurons cannot break down glucose completely. As a result, the energy balance is disrupted and ATP synthesis is deranged. Coupled with excitotoxicity mechanism, the influx of calcium leads to excess accumulation in the mitochondrial matrix which in turn leads to the opening of mitochondrial permeability transition pore (mtPTP) and the release of cytochrome c (X. Liu, Kim, Yang, Jemmerson, & Wang, 1996; Murphy, Fiskum, & Beal, 1999). These cellular events cause mitochondria to swell and lose its integrity, paving ways to cell death cascades such as apoptosis (X. Liu et al., 1996). As mentioned before, free radicals formed my mitochondria also contributes to cell death in ischemic environment (Kalogeris et al., 2014).

2.3 Risk Factors for Stroke

Identifying risk factors are crucial to inform stroke prevention strategies around the world and to reduce the global burden of stroke. There are numerous risk factors for stroke, comprising of both modifiable and non-modifiable risk factors. Non-modifiable factors include age, sex, race and genetics. The modifiable risk factors are of utmost importance, as health interventions should be geared towards changing lifestyle to reduce these factors which in turn reduces the risk of stroke. A retrospective hospital-based study in Malaysia has identified several controllable risk factors in stroke patients, including hypertension, diabetes mellitus, dyslipidemia, smoking and alcoholism (Boo et al., 2016).

Hypertension was found to be the major risk factor in stroke patients, in which, upon admission, the mean systolic blood pressure of the patients was 172.4 mmHg whereas the mean diastolic pressure was 93.8 mmHg in the aforementioned study. A recent prospective research conducted by Sepanlou et. al (2016) has found a significant association between hypertension and stroke mortality. These data indicate the profound role of hypertension in pathogenesis of cerebrovascular accident. High blood pressure causes a cascade of events, such as initiation of vasculopathy, formation of microatheroma, lipohyalinosis and atherosclerotic diseases, as well as disruption of blood-brain barrier (Hisham & Bayraktutan, 2013). Besides pharmacologic intervention, patients with hypertension are encouraged to involve in lifestyle changes, such as modification in diet and performing greater physical activity, in order to reduce the effects of hypertension (Chobanian et al., 2003). Intervention for hypertension, whether through medication or

behavioral changes, remains one of the most essential strategies in reducing the risk of cerebrovascular accident and its recurrence.

There is also a positive association between diabetes mellitus and incidence of stroke, particularly the ischemic subtype. Diabetes leads to several metabolic and pathologic changes that subsequently leads to stroke, including the stiffening of arteries, systematic inflammation, endothelial impairment and cardiac failure (R. Chen, Ovbiagele, & Feng, 2016; Quinn, Dawson, & Walters, 2011). With a proper dietary and weight management strategies, the prevalence of cardiovascular disease among diabetic patients can be lowered (Fox et al., 2015). This in turn reduces the risk of primary and secondary stroke encounter and the rate of mortality may decrease as well.

In addition to the study conducted by Boo et. al (2016), several other studies have pointed out that dyslipidemia is another risk factor that is associated with the occurrence of stroke (Sarti, Kaarisalo, & Tuomilehto, 2000; Tziomalos, Athyros, Karagiannis, & Mikhailidis, 2009). Dyslipidemia is defined as increased low-density lipoprotein (LDL) cholesterol levels, or low levels of high-density lipoprotein (HDL) cholesterol (Fodor, 2010). Atherosclerosis is the predominant mechanism of dyslipidemia, which eventually leads to cerebrovascular accident (Ansell, 2000). By decreasing the lipid profiles, the formation of atherosclerotic plaques can be reduced which in turn reduces the risk of stroke.

Cigarette smoking is a significant risk factor linked with stroke. An early research reported that smoking contributes approximately 15% of all stroke deaths per year (Thun,

Apicella, & Henley, 2000). Discontinuing smoking behavior is an approach to healthier lifestyle and researchers have identified that risk of stroke can be significantly reduced nearly 2 to 4 years after smoke cessation (Burns, 2003; Fagerström, 2002; Robbins, Manson, Lee, Satterfield, & Hennekens, 1994; Y.-M. Song & Cho, 2008). Besides, alcohol consumption also has effects on cerebrovascular accidents. A meta-analysis indicated that light to moderate alcohol consumption was inversely linked with ischemic stroke, whereas heavy drinking was associated with greater risk of all types of stroke with a stronger link with hemorrhagic stroke. The analysis signified that heavy drinkers were about 1.6 times more likely to suffer from intracerebral hemorrhage and 1.8 times are likely to suffer from subarachnoid hemorrhage (Larsson, Wallin, Wolk, & Markus, 2016).

Several potential modifiable risk factors that preceded cerebrovascular events have been highlighted. Reduction of risks of hypertension, diabetes mellitus, dyslipidemia, smoking behavior and alcoholism gradually decreases the risks associated with stroke. A healthy diet, frequent physical activity, smoking cessation, alcoholism restriction, coupled with medical interventions for hypertension, diabetes mellitus and dyslipidemia are found to be efficient strategies for stroke prevention (Boehme Amelia, Esenwa, & Elkind Mitchell, 2017).

2.4 Historic Antecedents of Transcranial Magnetic Stimulation (TMS)

Transcranial Magnetic Stimulation (TMS) is a non-invasive and painless armamentarium of stimulating certain cortical areas, and was developed by Dr. Antony T.

Barker in 1985. Following the invention, Dr. Barker won the first International Brain Stimulation Award for his outstanding contribution in the field of neuromodulation.

Dr. Barker initiated his research on utilizing time-differing magnetic fields to induce the flow of current in brain tissue in order to depolarize neurons. Prior to this research, the principal method used to induce neuronal depolarization is direct electrical stimulation that needs an attentive placement of the surface electrodes and attention on other aspects of the method to reduce discomfort among patients (Merton, Morton, Hill, & Marsden, 1982). This method was halted due several drawbacks and the high intensity of electrical stimulation often causes pain. On the other hand, the magnetic fields penetrate through the scalp and skull without being obstructed, and yields more precise results. In 1985, Dr. Barker and his colleagues recorded the first demonstration of TMS, in which applying TMS to the contralateral side of the motor cortex produced twitching in a specific area of the hand in human participants (Barker, Jalinous, & Freeston, 1985). This pioneer work has indicated the profound use of TMS as a pain-free method to activate specific area of brain.

2.4.1 Basic Principles of TMS

TMS was discovered using a fundamental idea on how magnetic fields and electric current interact with each other to modulate brain activity without putting patients at risk. It is essential to revise the connection between magnetic fields and electric current to fully understand the functions of TMS in treating neurological disorders. Faraday explained that electromagnetic induction happens when a transient current in copper coil yields a time-

differing magnetic field which induces a new electric current in nearby conductors (Faraday, 1832). A year after this discovery, Russian physicist, Heinrich Friedrich Emil Lenz reported that the direction of the electric current induced by the magnetic field is opposite to the electric current flow that produces it. This phenomenon was called "Len's law" (Lenz, 1834).

Putting all these facts together, TMS administers a transient electric current that flows through a magnetic coil positioned on the scalp, producing a time-varying magnetic field which in turn induces electric current flow in cortical neurons, consistent with the "law of electromagnetic induction and the Lenz's Law" (Figure 2.3).

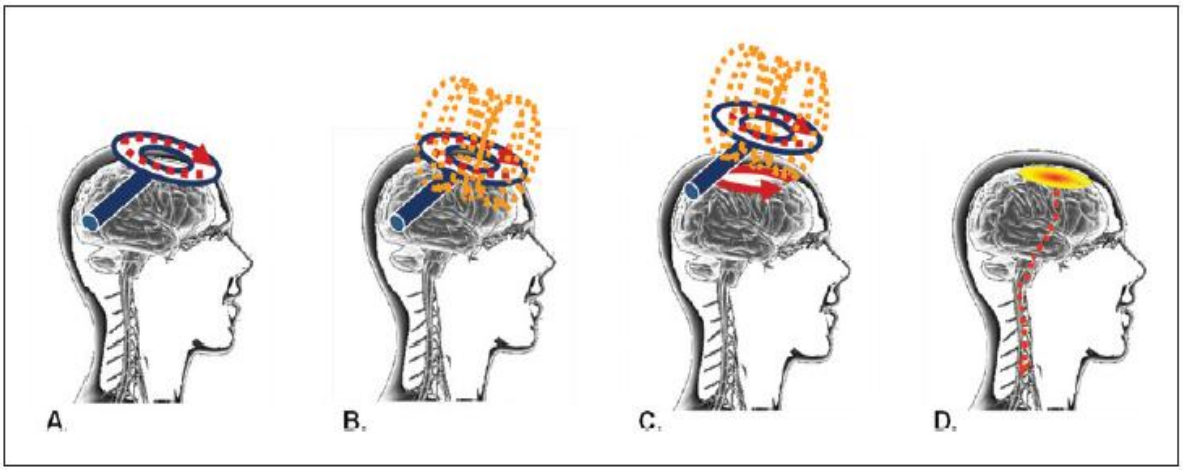

Figure 2.3: The working principles of TMS. (A) Electric current flows through electromagnet coil. (B) Time-varying magnetic field is yielded by electric current flowing through the coil. (C) Electric current induced by the magnetic field is opposite to the electric current flow produced by coil. (D) Depolarization of neurons stimulates the corticospinal tract during TMS. Source: (Vidal Dourado et al., 2013).

TMS can be administered through different kinds of coils, including those in circular shapes, double cone coil and "figure-of-eight" coils with diameters ranging from 40 to 90 mm (T. Wagner, Valero-Cabre, & Pascual-Leone, 2007). It has been indicated that the flux of the magnetic field produced by the figure-of-eight coil is more concentrated and more focused when compared to simple circular coils. TMS pulse in the figure-of-eight coil reaches a depth of 1.5 to 3 cm (Rossi, Hallett, Rossini, & Pascual-Leone, 2009; Thielscher & Kammer, 2004; Zangen, Roth, Voller, & Hallett, 2005). On the other hand, TMS for subcortical areas can be done using H-coil (Bersani et al., 2013).

Since its discovery, TMS has undergone rapid advancements in which, in recent years, TMS can be neuronavigated to stimulate certain parts of the cortex and associated with other neuroimaging techniques such as electroencephalogram (EEG), functional magnetic stimulation (fMRI), positron emission tomography (PET) and transcranial Doppler ultrasonography (Ahdab, Ayache, Brugieres, Goujon, & Lefaucheur, 2010; Bashir, Edwards, & Pascual-Leone, 2011; Denslow, Lomarev, George, & Bohning, 2005; Julkunen et al., 2009; Sack & Linden, 2003; Sparing, Buelte, Meister, Pauš, & Fink, 2008; Thut & Pascual-Leone, 2010).

2.4.2 Methods of TMS

There are two common methods of TMS, which are single-pulse (including paired-pulse) and repetitive TMS. Paired-pulse TMS comprised of two consecutive pulses being administered through the same electromagnet coil, with either a short inter-stimulus interval of a few milliseconds or a long inter-stimulus interval of tens to hundreds of

milliseconds. This neurostimulation technique is utilized to explore inhibitory or excitatory cortical networks depending on the intensity of stimulation and inter-stimulus interval being used (Kujirai et al., 1993; Tokimura, Ridding, Tokimura, Amassian, & Rothwell, 1996; Valls-Solé, Pascual-Leone, Wassermann, & Hallett, 1992). These two pulses can also be applied over each hemisphere at the same area of the motor cortex in order to examine inter-hemispheric inhibition (Ferbert et al., 1992).

On the other hand, repetitive TMS (rTMS) is a neuromodulation technique that modulates cortical activity beyond the period of stimulation, and acts as a therapeutic tool for the treatment of neurological and psychiatric disorders. Repetitive TMS protocols include simple techniques such as low-frequency and high-frequency stimulations, as well as new modulatory approaches that include theta burst stimulation and paired associative stimulation. Each of this protocol has its own procedures and the outcomes differ from each other, depending on the frequency of stimulation and the duration of stimulation period (Simonetta-Moreau, 2014).

In studies that utilize low-frequency rTMS protocol, the stimulation frequency is usually set at 1 Hz, and scrutinized to have inhibitory effect. However, at low frequencies (lower than motor threshold), 1 Hz stimulation often fails to exhibit considerable effects on corticomotor excitability. In contrary, high frequency rTMS (5-25 Hz) enhances corticomotor excitability, and has received attention because it is an essential intervention for the recovery of upper-limb motor nerve function among stroke patients (Higgins, Koski, & Xie, 2013b; Sasaki, Mizutani, Kakuda, & Abo, 2013). Due to promising outcomes in

previous research, current research aims to demonstrate the effectiveness of 10 Hz rTMS protocol by examining the changes in musculoskeletal biomarkers among stroke patients.

The new rTMS methods include theta burst stimulation (TBS) and paired associative stimulation (PAS). Similar to simple rTMS methods, TBS has two different patterns of stimulation that increases and depresses the corticomotor excitability. An intermittent TBS (iTBS) pattern is applied for 2 s and then repeated every 10 s, results in enhancement of corticomotor excitability (V Di Lazzaro et al., 2010; Y.-Z. Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005; Simonetta-Moreau, 2014). On the other hand, continuous TBS (cTBS) is a form of rTMS that depresses corticomotor excitability when it is delivered for 40 s without any interval. Researchers assume that TBS generates both fascilitatory and inhibitory effects, with facilitation occurs faster than inhibition (Y.-Z. Huang et al., 2005).

Another approach of newer rTMS is termed paired associative stimulation (PAS). PAS was built using principles employed by associative LTP or the Hebbian concept described in animal studies. The general idea of the aforementioned concept is that the temporal order of the presynaptic and postsynaptic spiking determines whether LTP and LTD is induced when a weak and strong input are stimulated together (Levy & Steward, 1983). PAS also exhibits fascilitatory and inhibitory effects on the excitability of the motor cortex in humans, depending on the inter-stimulus interval (ISI) between electrical peripheral nerve stimulation and cortical stimulation. If the ISI is shorter than the afferent delay (time required for the peripheral afferent input to reach the brain), PAS decreases corticomotor excitability. In contrary, if ISI is longer than the afferent delay, PAS increases

cortical excitability (Wolters et al., 2003). Neurophysiologic studies additionally describe the role of NMDA and $GABA_B$ receptors that cause the effects of PAS. It has also been proven that dopamine could also be responsible for inducing PAS after-effects (Hoogendam, Ramakers, & Di Lazzaro, 2010). For a clearer description, the types and properties of different patterns of rTMS are summarized in Figure 2.4.

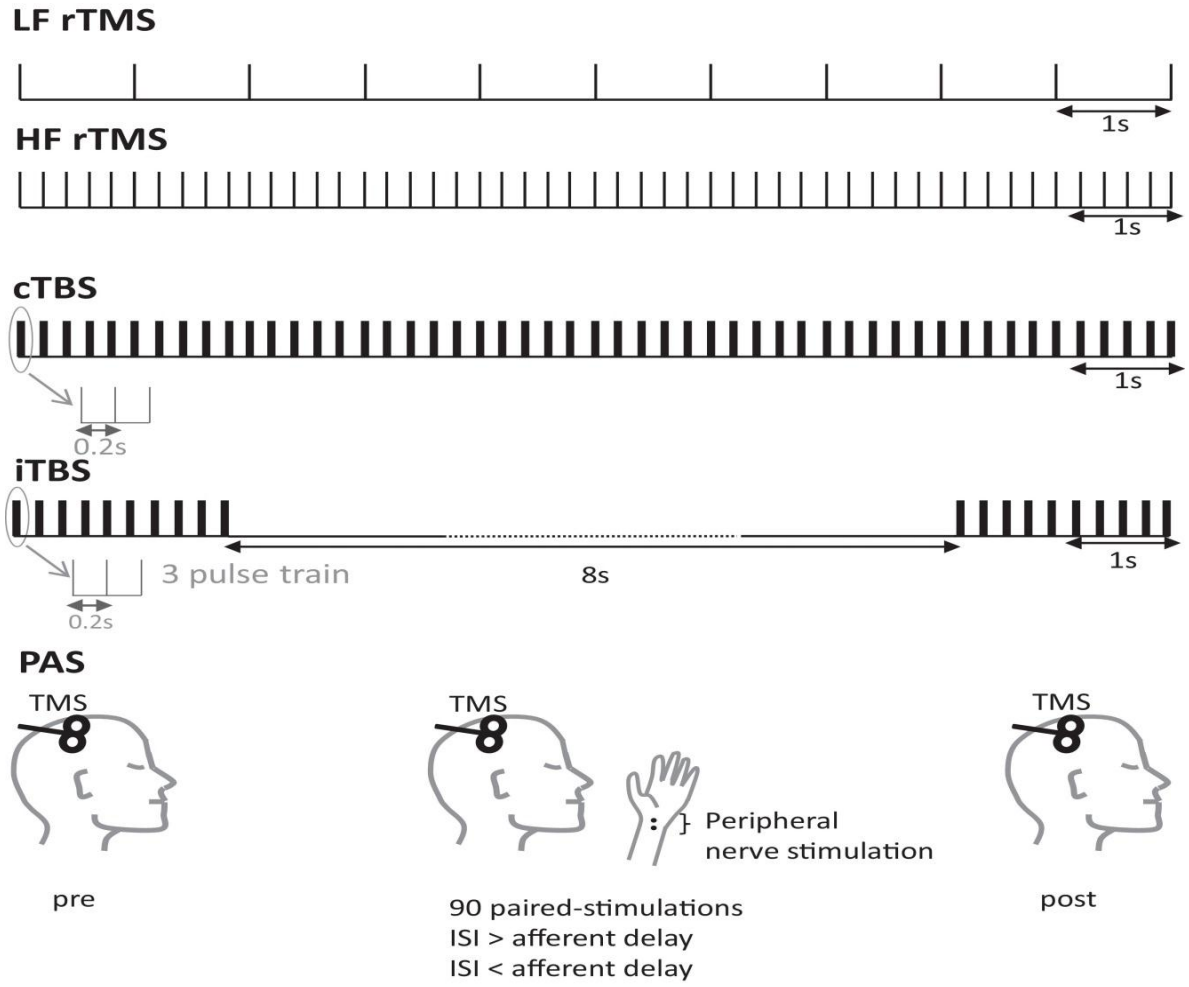

Figure 2.4: Simple repetitive TMS (rTMS) protocols consist of similar stimuli spaced by an identical inter-stimulus interval (ISI). The effects of simple rTMS depend on stimulation frequency: low frequency (< 1 Hz) rTMS depresses cortical excitability, whereas high frequency (> 5 Hz) increases cortical excitability. Theta burst stimulation (TBS) protocol