UNIVERSIDADE DE SÃO PAULO
INSTITUTO DE PSICOLOGIA

CAMILA BONIN PINTO

Efeito da terapia combinada da EMTr com fluoxetina na reabilitação da função motora de pacientes pós AVE isquêmico

Effects of contralesional repetitive magnetic stimulation combined with fluoxetine on motor recovery in stroke patients

São Paulo
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Dedico este trabalho à minha família,

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Spaulding Rehabilitation Network
Research Institute
79/96 Thirteenth Street
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Charlestown, MA 02129

www.spauldingrehab.org

Initial Review: Notification of IRB Approval/Activation
Protocol #: 2014P001046/SRH

Date: August 21, 2014

To: Felipe Fregni, MD, Ph.D
SRH
Dept of Physical Med and Rehab

From: Spaulding Rehabilitation Network Research Institute
79/96 Thirteenth Street
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This project has been reviewed by SRH IRB . During the review of this project, the IRB specifically considered (i) the risks and anticipated benefits, if any, to subjects; (ii) the selection of subjects; (iii) the procedures for obtaining and documenting informed consent; (iv) the safety of subjects; and (v) the privacy of subjects and confidentiality of the data.

Please note that if an IRB member had a conflict of interest with regard to the review of this project, consistent with IRB policies and procedures, the member was required to leave the room during the discussion and vote on this project except to provide information requested by the IRB.

Approved Documents

Protocol Summary (version date: 08/19/2014)
Detailed Protocol (version date: 08/19/2014)
Consent Form (version date: 08/13/2014)
Telephone Script
Beck Depression Inventory
HAM-D
Demographic Data

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Modified Ashworth Scale
Modified Rankin Scale
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Medical History Form
Emergency Procedures 79/96
Designation Letter

As Principal Investigator, you are responsible for ensuring that this project is conducted in compliance with all applicable federal, state and local laws and regulations, institutional policies, and requirements of the IRB, which include, but are not limited to, the following:

1. Submission of any and all proposed changes to this project (e.g., protocol, recruitment materials, consent form, status of the study, etc.) to the IRB for review and approval prior to initiation of the change(s), except where necessary to eliminate apparent immediate hazards to the subject(s). Changes made to eliminate apparent immediate hazards to subjects must be reported to the IRB as an unanticipated problem.
2. Submission of continuing review submissions for re-approval of the project prior to expiration of IRB approval and a final continuing review submission when the project has been completed.
3. Submission of any and all unanticipated problems, including adverse event(s) in accordance with the IRB's policy on reporting unanticipated problems including adverse events.
4. Obtaining informed consent from subjects or their legally authorized representative prior to initiation of research procedures when and as required by the IRB and, when applicable, documenting informed consent using the current IRB approved consent form(s)with the IRB-approval stamp in the document footer.
5. Informing all investigators and study staff listed on the project of changes and unanticipated problems, including adverse events, involving risks to subjects or others.
6. When investigator financial disclosure forms are required, updating your financial interests in Insight and for informing all site responsible investigators, co-investigators and any other members of the study staff identified by you as being responsible for the design, conduct, or reporting of this research study of their obligation to update their financial interest disclosures in Insight if (a) they have acquired new financial interests related to the study and/or (b) any of their previously reported financial interests related to the study have changed.

The IRB has the authority to terminate projects that are not in compliance with these requirements.

Questions related to this project may be directed to Catherine E Sutherland, CSUTHERLAND1@PARTNERS.ORG, 617-952-6182.

RESUMO

Pinto, CB. Efeito da Terapia combinada da EMTr com fluoxetina na reabilitação da função motora de pacientes pós AVE isquêmico. São Paulo. Instituto de Psicologia da Universidade De São Paulo,2018

O AVC está entre a principais causas de mortalidade e disfuncionalidade no mundo. A recuperação da função motora pós-AVC é normalmente incompleta; uma vez que as terapias atuais tem impacto limitado na promoção da plasticidade cerebral. Novas abordagens que podem intensificar a plasticidade cerebral têm sido estudadas para melhorar a reabilitação motora pós-AVC, entre eles a fluoxetina e a estimulação magnética transcraniana (EMTr) alcançaram resultados promissores. Portanto, nós conduzimos um ensaio clínico exploratório randomizado, duplo-cego, placebo controlado, avaliando os efeitos da combinação da EMTr em baixa frequência com a fluoxetina para aumentar a função motora do membro superior em pacientes com AVC. Vinte e sete pacientes hemiplégicos secundários a AVC isquêmico que apresentaram o evento nos últimos 2 anos foram randomizados em três grupos: EMTr ativa + fluoxetina, sham EMTr + fluoxetina e placebo (sham EMTr + fluoxetina placebo). Os participantes receberem 18 sessões (10 sessões diárias seguidas de 8 sessões semanais) de EMTr a 1 Hz sobre o córtex motor primário (M1) do hemisfério não afetado, combinadas com 90 dias de fluoxetina (20 mg/dia). As escalas de Jebsen Taylor (JTHF) e Fugl-Myer (FMA) foram utilizadas. Além disso, desfechos secundários incluíram questionário de segurança e comportamentais. Nossos resultados demonstraram melhora significativa na FMA e JTHF após o tratamento nos três grupos. Após ajustar para o tempo desde o evento isquêmico houve um aumento significativo na melhora da função motora de acordo com o JTHF no grupo que combinou EMTr ativa + fluoxetina quando comparados os grupos placebo ou fluoxetina exclusivamente. Essa análise mostrou uma melhora menos significativa na função motora no grupo fluoxetina quando comparada com o grupo placebo quando avaliada pelo JTHF ($p=0.038$) e pelo FMA ($p=0.039$), sugerindo um efeito potencialmente prejudicial da medicação ativa quando comparada com o placebo. Por fim, observamos que os desfechos de humor, função cognitiva e a segurança não foram significativos. A combinação da EMTr com a fluoxetina demonstrou melhoras significativas na função motora pós-AVC quando comparada com placebo, a terapia exclusiva com fluoxetina parece causar um efeito negativo.

Palavras-chave: acidente vascular cerebral, estimulação magnética transcraniana, fluoxetina, função motora

ABSTRACT

Pinto, CB. Effects of contralesional repetitive magnetic stimulation combined with fluoxetine on motor recovery in stroke patients. São Paulo. Instituto de Psicologia da Universidade De São Paulo,2018

Stroke is among the leading causes of mortality and disability worldwide. Post stroke recovery of motor function is usually incomplete; these poor effects are believed to be due to the limited impact of current therapies in promoting brain plasticity. Novel approaches that can enhance brain plasticity have been studied to improve motor rehabilitation after stroke, among them fluoxetine and repetitive transcranial magnetic stimulation (rTMS) yielded promising results. Therefore, we conducted a randomized, double-blinded, sham-controlled, exploratory trial evaluating the effects of the combination of low-frequency rTMS and fluoxetine to increase upper limb motor function in stroke patients. Twenty-seven hemiplegic ischemic stroke patients within 2 years post event were randomized into three groups: active rTMS+fluoxetine, sham rTMS+fluoxetine, or placebo (sham rTMS+ placebo fluoxetine). Participants received 18 sessions (10 daily sessions followed by 8 weekly sessions) of 1Hz rTMS applied over the primary motor cortex (M1) over the unaffected hemisphere combined with 90 days of fluoxetine (20 mg/day). A blinded rater assessed motor function as indexed by Jebsen Taylor hand function (JTHF) and Fugl-Myer (FMA) scales. Additional secondary outcomes included safety and behavioral questionnaires. Our results showed a significant improvement in FMA and JTHF post treatment in all three groups. After adjusting for time since stroke there was a significantly larger improvement in motor function as indexed by JTHF seen in the combined active rTMS+fluoxetine group when compared to placebo and fluoxetine only groups. Additionally, this analysis showed significant less improvement in motor function in the fluoxetine group when compared to placebo group as indexed by JTHF ($p=0.038$) and FMA ($p=0.039$); consequently, suggesting a potential detrimental effect of the active medication when compared to placebo. Lastly, we observed that mood, cognitive performance and safety outcomes were not significantly. Despite establishing that the combination of TMS and fluoxetine leads to higher/greater improvements in motor function post stroke when compared to placebo, solely therapy with fluoxetine seemed to lead to a negative effect and thus it is plausible to believe that the benefit observed in the combined group is more likely due to the effects of TMS intervention.

Key-words: stroke, transcranial magnetic stimulation, fluoxetine, motor function

List of Figures

List of Tables

Table of Contents

1. INTRODUCTION

In this introduction, preclinical and clinical evidence on the use of selective serotonin reuptake inhibitors (SSRIs) and Transcranial magnetic stimulation (TMS) in stroke motor function recovery is reviewed. We collected this evidence as to discuss the following important issues: (i) Stroke and plasticity after stroke (ii) mechanisms of SSRIs; (iii) effects of these drugs in brain plasticity as shown in previous studies; (iv) whether there is a clinical effect in stroke; (v) mechanism of rTMS (vi) whether there is a clinical effect in stroke (vii) whether there are interactions/synergistic effects on plasticity between SSRIs and TMS, and whether they are depending on the time since stroke and severity of lesion; (viii) combination of SSRIs with non-pharmacological treatments in stroke. Understanding these issues can help in the development of novel therapeutic approaches for stroke recovery and facilitate the interpretation of the results of this trial.

1.1. Stroke

Stroke is the second most common cause of death worldwide (1) and the global leading cause of disability (2), about 6.7 million people died of stroke in 2012. According to the American Heart Association, approximately 800,000 people have a stroke every year in United States (US) and 87% of those are classified as ischemic stroke (1), caused by a blockage of the supply of the blood vessels in the brain. The costs for continued care for stroke survivors is approximately $38.6 billion annually. In Brazil, there are nearly 68,000 deaths/year from stroke and it is the condition that represents the first cause of death and incapacity in the country, generating great economic and social impact According to the Ministry of Health's Health Portal (2012), 79,185 admissions were secondary to a stroke in 2011, which cost R$ 197.9 million for the Unified Health System.

Stroke is defined by the World Health Organization (as an acute episode of neurological dysfunction secondary to an ischemia or hemorrhage, with clinical signs of focal or global disturbance of brain function. The inadequate blood supply leads to an impairment of brain functions due to parenchymal and neural pathway disruption. In the hemorrhagic the interruption of blood supply can be caused by a blood vessel rupture followed by extravasation of blood into the surroundings of the brain parenchyma/tissue and in the ischemic is caused by a blockage in a

vessel. In both situations, there is lack of oxygen and nutrients supply which causes damage to the brain tissue altering its function.

Even thought, reperfusion therapies in ischemic stroke (i.e. thrombolysis and thrombectomy) can restore blood supply and improve neurological outcomes (3, 4), they are not always effective and between 55 to 75% of stroke survivors will still have functional limitations at 3-6 months after the event (5, 6).

One of the most common sensory-motor sequelae is the partial loss of motor function in the upper limbs, which limits the performance of the affected extremity (6, 7). Most stroke patients are unable to perform activities of daily living or professional duties affecting the patient's daily life performance.

Many decades back, it was believed that the sequalae or symptoms seen in patient after brain injuries – including stroke – were irreversible. However, at bedside stroke survivors were improving spontaneously after the event. In recent years, our understanding of post stroke plasticity and spontaneous recovery has grown significantly, improving research on rehabilitation. To better understand how to modulate brain plasticity after a stroke the next session will discuss plasticity phenomena and brain organization processes occurring after a stroke, as well as the mechanism of recovery in acute and chronic stroke.

1.2. Plasticity after stroke

Following stroke, different structural changes take place leading to acute neurological deficits. The injured brain region suffers a lack of oxygenation that can lead to neurodegeneration through an increased apoptotic rate and accelerated inflammatory processes. The areas more affected are localized closest to the affected vessel. Nonetheless, remote brain areas can be affected by the disruption of additional networks and connectivity. Such widespread distress may have a significant role in the sensitive, motor and cognitive dysfunctions seen after the injury, thus setting a pace for post-stroke rehabilitation (8). Additionally, the degree of damage is as well dependent on the amount of collateral blood supply of the affected region.

In 1975, based on his own experience after suffering a stroke, the neuroanatomist Alf Brodal stated: *“Since regeneration of transectioned central axons has never been convincingly*

demonstrated in higher mammals, it seems in most instances that one must resort to the assumption that ***intact fibers take over for the damaged ones****.*" Indeed, early after stroke several neuro-protective changes involving biochemical cascades can take place (e.g., resolution of cerebral edema, absorption of the damaged tissue, inflammation and changes in neurotransmitters regulation levels) (8, 9). Apart from these neuro-protective responses, series of spontaneous events such as the unmasking of previous connections and the reduction in inhibition leads to very rapid changes in cortical mapping and synaptic plasticity.

1.3. Disruption of Inhibitory Activity after Stroke

In this context, different research groups have been investigating brain activity and cortical plasticity of stroke patients during recovery and comparing it with healthy subjects. In a healthy brain, neural activity of motor areas from the two hemispheres is functionally coupled by a mutual equally balanced inhibitory control - by transcallosal circuits - known as inter-hemispheric inhibition. This neurophysiologic mechanism allows one hemisphere to inhibit the opposite one during unilateral movements (10). After a stroke, this balance can be affected, generating a maladaptive pattern of over-activation (lack of inhibition) in the non-lesioned motor cortex, which exerts a pathological inhibition over the injured hemisphere (11). Such changes induced by stroke are considered as possible causes of the increased cortical excitability found in the contralesional hemisphere and the reduced cortical excitability in the injured hemisphere (12).

The interactions between excitatory and inhibitory activity in learning-related circuits are crucial to drive motor cortex plasticity and recovery after stroke (13). In the very acute phase of stroke (hours after the event), a reduction of activity of cortical areas surrounding the lesion is required since increased excitability in these areas can result in glutamatergic toxicity and increase apoptosis (14, 15).

After this initial phase - and similarly to early stages of learning - the excitation/inhibition ratio increases (by simultaneously or independent decrease of inhibition and/or increase in excitation levels) (14). This is a neuroprotective phenomenon that takes place few hours after injury and is part of longitudinal neuroplastic changes that results in reconfiguration of motor networks

through the establishment of alternative inputs for the cortico-spinal tracts. This activates parallel motor circuits and transfers the impaired functions to unaffected areas of the brain (16-19).

These changes in the ratio of excitation and inhibition have been associated with brain remodeling and recovery after stroke:

(i) an initial increase in excitability in perilesional areas
(ii) increased activity in the contralesional hemisphere.

In this context, several studies showed that in stroke patients the inhibitory function is globally reduced in both hemispheres suggesting that the modulation of inhibitory circuits might result from a strategy that aims at compensating for motor impairment (20, 21). Even though this enhanced excitability pattern is related to the increased structural plasticity and functional reorganization, both changes are required to be time dependent to have a beneficial effect in stroke recovery (12).

Therefore, stronger functional recovery is associated with a time-dependent modulation of inhibition - strong inhibition close to the event and a decrease of inhibition over time. In fact, an important aspect that has not been fully explored in human studies is the modulation of the inhibitory states; varying from disinhibition to inhibition in cortico-subcortical circuits according to the phase of learning. A shift in one direction only (i.e., disinhibitory state) has a low learning efficiency (12). In fact, that shift may be an index of cortical disorganization. However, the inhibition/excitation state is hard to be measured and most of the times not taken into account during the stroke recovery process.

To date, the mechanisms behind motor impairment, cortical excitability and recovery after stroke are still not fully understood. So far, neither changes in cortical excitability (Motor evoked potential - MEP), intrahemispheric facilitation and inhibition (ICF and ICI), or interhemispheric inhibition can be applied as models to explain stroke recovery and responses to treatment. Moreover, stroke rehabilitation involves a much more complex and multimodal system since these effects of inhibition and disinhibition between hemispheres depend as well on the degree of impairment, lesion localization and stroke size (22).

Considering the prediction of an increased number of stroke patients by 2030 (23), there has been intensive research on therapies to enhance neuroplasticity and motor learning in stroke. Notwithstanding, current conventional therapies rely on behavioral treatments such as physiotherapy and occupational therapy inducing limited plastic and cortical reorganization changes (24, 25).

As previously mentioned, the principles of motor learning after a stroke involve neuronal excitability modulatory mechanisms that are very similar to the ones involved in memory and learning processes (26). Recent studies have been developed to evaluate the effects of drugs capable of modulating neural excitability and consequently modifying learning and memory processing. Some of these pharmacological candidates are selective serotonin reuptake inhibitors (SSRIs)(27-29), dopamine agonists (30), amphetamines (31) and cholinergic substances (ACR)(32).

Out of these, SSRIs are the most promising candidate to drive plasticity and enhance motor function after stroke. In 2011, the FLAME trial (28) showed the role of fluoxetine combined with physiotherapy in upper limb motor rehabilitation, indexed by the Fugl-Meyer Motor Scale (FMA), in acute ischemic stroke patients who were prescribed 20 mg of fluoxetine for three months, starting 5-10 days after the event onset.

However, it is still not clear how this class of medications can enhance neuroplasticity or whether its positive effects on stroke motor recovery are mediated by other pathways that do not involve neuroplasticity (33).

1.4. Mechanisms of SSRIs

The role of SSRIs in motor function recovery after stroke remain as a topic of discussion, since different clinical trials and animal models yielded inconsistent results. SSRIs block serotonin (5-HT) reuptake and increase the availability of this neurotransmitter in the synaptic cleft; therefore, enhancing serotonergic synapse and signal transmission (34). Consequently, increasing the excitatory input of glutamate (resulting from the serotonergic synapse), activating NMDA receptors which leads to a cascade of intracellular events. These events might generate synaptic modifications that culminate in an long term potentiation – (LTP)-like effect leading to synapse reprogramming and strengthening (35).

Although this model is generally accepted as the main mechanism of SSRIs, this is simplistic and does not consider adaptations to the dynamic biochemical neural environment. One possibility is that the increase of 5-HT in the synapses can results in a negative feedback leading to a decrease of 5-HT release; culminating in compensatory effect (34, 35). It seems on the other hand that chronic use may overcome this compensatory effect and lead to a normalization and to further increase in 5-HT levels (36). Given the uncertainty of the neurochemical model of serotonin, it is important to understand its direct effect on neuroplasticity. Bellow we summarize the possible effects of SSRIs in brain plasticity (

Figure 1)

1.4.1. The effects of SSRIs in modulating brain plasticity

Cortical plasticity after stroke occurs through several mechanisms including the release of neurotransmitters. In this context, SSRIs may play a role in enhancing overall brain plasticity by increasing serotonin dependent synapses (34). Besides that, recent studies have been showing the influences of fluoxetine in critical biological pathways in stroke survivors as discussed in the following subsections.

1.4.2. Growth factors and cerebral blood flow regulation

Animal studies showed the effects of fluoxetine over blood flow regulation and angiogenesis. Shin (37) and collaborators showed that acute fluoxetine treatment after an ischemic stroke (for 14 days, starting 1 hour after the event) generated a reduction in the infarct size, restored cerebral blood flow (CBF) autoregulation, and elevated the expression of hypoxia-inducible factor-1α (HIF-1α) and of heme oxygense-1 (HO-1) (37). HIF-1α favors neuronal survival after ischemia (38, 39) and the induction of HO-1 protein may protect cerebral tissue from ischemic damage. Moreover, the effects of fluoxetine over CBF regulation were demonstrated regardless the presence of brain lesions, showing potential to promote cell regeneration and repair in later stages of rehabilitation (27, 40).

In addition, some studies demonstrated increased angiogenesis following fluoxetine treatment, specifically, by increasing vascular endothelial growth factor (VEGF) levels. This growth factor is associated with neuroplasticity, since it plays a role in reestablishing vascular networks after hypoxia and ischemia, which is essential for neuronal cell proliferation and growth (41-43). Pre-clinical trials showed that, after focal ischemia, treatment with VEGF reduced infarct size, improved neuronal cell survival and stimulated angiogenesis (44). The effects of VEGF on angiogenesis were observed not only after ischemic lesions, but also in healthy brains (45) and depressed patients treated with antidepressants (including SSRIs) (46).

1.4.3. Neurogenesis

Different studies have been showing the neuro-histological effects of antidepressants, Malykhin and collaborators observed an increased size of the hippocampus body in major depressive patients treated with antidepressants when compared with non-treated patients. Additionally, it has been established that SSRIs can promote neuronal sprouting and re-hardwiring (47, 48).

In the same way, animal studies have shown that adult neurogenesis also occurs in several brain regions outside of the hippocampus. Most notably in periventricular areas surrounding the third ventricle, including the hypothalamus (49) and circumventricular organs (50).

Likewise, Ohira and coworkers (51) showed that in adult rats fluoxetine treatment - after an induced stroke lesion - increased the number of a subset of interneurons generated from Layer 1 inhibitory neuron progenitor cells (L1-INP cells). The increase in the number of L1-INP cells after 3 weeks of treatment varied upon brain region and was dose-dependent. Among cortical regions, the increase in the number of L1-INP cells was most prominent in the frontal cortex, with a significant increase at 3 weeks. The administration of fluoxetine led to an increase in the number of GABAergic interneurons in the cortex at 4 weeks after the last fluoxetine injection. This potential neurogenesis-driven mechanism is independent of brain lesion occurrence and it seems to be independent of time since lesion occurrence. However, the presence of L1-INP cells have not been reported in other mammals.

1.4.4. Cortical excitability

SSRIs can be compensating some of the neurophysiological damage caused by stroke by promoting neuroplastic changes. Besides that, it is essential for motor learning since the 5-HT system plays an important role in the sensory motor synapses; promoting excitability of the spinal motor neurons and in the adrenergic system since it can up regulate β-adrenergic receptors.

Studies have shown that a single dose of a serotonergic drug alters cortical excitability (52). Paired associative stimulation (PAS) is a non-invasive brain stimulation (NIBS) technique capable of promoting changes in cortical excitability of the motor system and is one of the most studied approaches to induce activity-dependent cortical stimulation (53). In PAS, a low frequency repetitive electric stimulation of the median nerve is combined with a delayed transcranial magnetic stimulation (TMS) stimuli over the corresponding primary motor cortex (M1) (53). The LTP/LTD-like plasticity effects are dependent on the interstimulus interval between median nerve stimulation and the TMS pulse. In order to explore the effects of SSRIs in PAS-generated alterations in cortical excitability, the same research group treated 14 healthy subjects with a single dose of citalopram and following a cross-over design performed inhibitory and excitatory PAS (Table 1). Similar effects were observed in both groups, showing that the administration of a single dose of citalopram hindered the after-effects of inhibitory PAS – since the group receiving inhibitory PAS plus citalopram also had increased cortical excitability – and enhances the after-effects of excitatory PAS (54).

Furthermore, a study by Nitsche and collaborators showed that a single dose of citalopram (20 mg) enhanced plastic changes promoted by excitatory (anodal) transcranial direct current stimulation (tDCS) (55). Besides that, when the subjects received inhibitory tDCS (cathodal), the SSRIs medication changed the response pattern from inhibitory to a facilitatory stimuli (54). Therefore, SSRIs were shown to modulate the changes in cortical excitability promoted by tDCS, resulting in an enhanced LTP-like plasticity (citalopram plus anodal tDCS).

1.4.5. Neuroprotective mechanism

A systematic review and meta-analysis published in 2014 showed that antidepressant treatments including SSRIs can decrease infarct volume and improve neurobehavioral outcomes in animal

models of induced ischemic stroke (56). The neuroprotective effects of fluoxetine and other SSRIs seem to be related with its ability to inhibit later post-ischemic inflammatory pathways, such as microglia and neutrophils NF-κB activation (57, 58). On the other hand, another recent preclinical study showed that the positive effects of fluoxetine on motor recovery are independent of mechanisms related to inflammation, such as neuronal death and infarct volume (59).

1.4.6. Fluoxetine enhancing motor cortex inhibition

Recent studies suggest that fluoxetine may affect the balance between excitation and inhibition after stroke, more specifically modifying inhibitory pathways. Previous insights from its role in the treatment of major depressive disorder suggest that fluoxetine can restore the impaired inhibition profile, commonly observed in depression (60, 61). Moreover, some preclinical data showed that fluoxetine interacts with the GABAergic system, culminating in enhanced inhibitory tonus (62-64). In the stroke context, intercortical imbalance as well as the disinhibition observed in both hemispheres plays a role during the rehabilitation. The lack of inhibition in the unaffected hemisphere can be associated with poor motor recovery. Therefore, fluoxetine can reestablish the balance between excitation and inhibition after stroke leading to improvement in motor function (12).

The brain possesses the intrinsic capability to develop new connections and strengthening as well as weakening existing ones to acquire new functions to compensate for impairment. These processes are independent of the extension and timing of any lesion; therefore, it can be enhanced and redirected during any phase of rehabilitation (11, 65, 66). To date, several research studies observed neuroplastic changes, by showing that recovery of function after stroke is in part independent of spontaneous recovery associated with the acute phase (67, 68). Different authors (69, 70) have clearly stated that motor learning and neuroplasticity (71, 72) are evident even in late stages after stroke.

In accordance to that, animal's studies analyzing the effects of SSRIs in motor recovery showed positive results regardless of the stroke phase and lesion severity (56, 73-75), as well as that neural plasticity is crucial for motor recovery after a stroke (11).

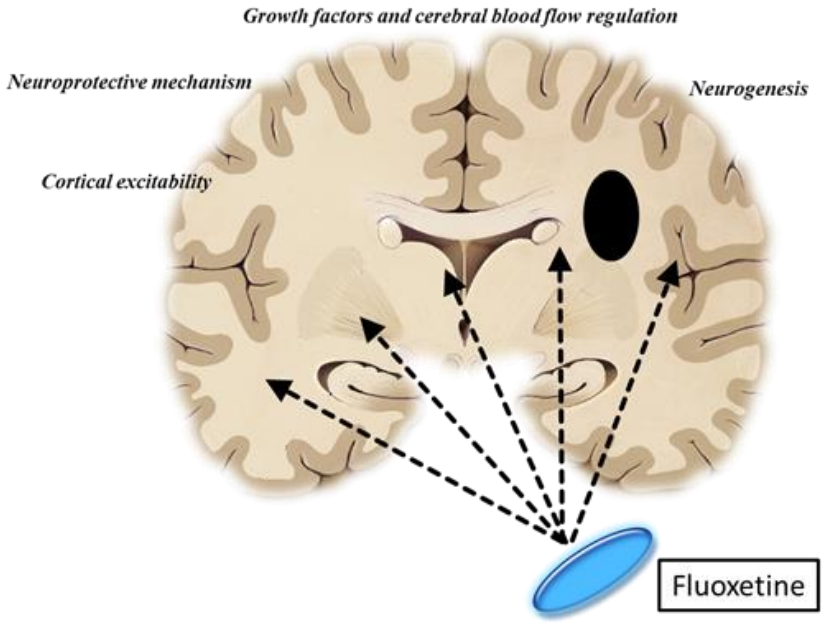


Figure 1*:* SSRIs possible mechanisms.
Summary of SSRIs possible mechanism altering brain plasticity and contributing to a "better" outcome after stroke.

1.5. Clinical evidence of SSRIs on motor recovery after stroke

Several studies have suggested that the serotonergic agent's fluoxetine and citalopram have a positive effect on motor function in hemiplegic patients after stroke (27, 28, 75-78).

To date, the vast majority of clinical trials studying the effects of SSRIs on stroke evaluates them in the context of post-stroke depression (79). According to recent studies between 30 to 50% of the stroke patients will develop depression during some period of their rehabilitation process (80). In 2013, Mead and collaborators performed a Cochrane systematic review for stroke recovery, including 52 trials and 4059 patients (79). The meta-analyses reported that patients allocated in the SSRIs groups were less dependent, disabled, neurologically impaired, depressed or anxious at the end of the treatment. However, most of the studies assessing the role of SSRIs in stroke recovery used global function or dependency scale, and no difference was found when analyzing motor function by more specific scales such as the Fugl-Meyer Assessment (FMA).

Currently, the FLAME trial (28) is the largest placebo-controlled clinical study developed to evaluate the effects of a SSRIs on motor recovery after stroke. The trial measured the effects of

20 mg fluoxetine once a day for 3 months combined with physical therapy using a clinical outcome specific for motor recovery. Its results, published in 2011, showed a moderate effect size FMA for upper and lower limbs in the fluoxetine group when compared with the placebo group (28). This trial increased the interest and use of fluoxetine after stroke motor recovery.

In summary, although the FLAME study and the Cochrane review showed promising results, the effect of SSRIs in stroke recovery independent of depression has not yet been established and demand further investigation (79). Even though none of these studies evaluated the specific role of the SSRIs in stroke recovery, as discussed previously, some insights from animal models showed that SSRIs can influence the rate and degree of post-stroke recovery lesions since it can modulate important cellular plasticity, cell growth and proliferation.

1.6. Association of SSRIs with non-pharmacological treatments in stroke

As discussed in previous sections, targeting the maladaptive pattern of over-activation (lack of inhibition) in the non-lesioned motor cortex after a stroke can be a successful tool to enhance motor rehabilitation. In one hand, fluoxetine has been showing potential to induce neuroplastic changes compensating some of the neurophysiologic damage caused by a stroke. On the other hand, it has been recognized the effect of noninvasive brain stimulation (NIBS) techniques have shown promising results in stroke motor rehabilitation by targeting the inhibition imbalance in the motor cortex (81-84).

In this context, it seems plausible to combine the mechanistic properties of both therapeutic approaches, to add synergism in their effects on neuromodulation and function restoration. In the next session, we will discuss TMS and its role on stroke rehabilitation.

1.7. Transcranial Magnetic Stimulation

In recent years, the number of scientific research and possible clinical indications of NIBS increased. Among NIBS techniques, TMS is a highly effective way to non-invasively modulate and measure cortical excitability that has been studied and applied since 1985. However, the path towards noninvasive treatment using electricity has evolved over a large amount of time and required technological advances and intensive clinical research. This session covers several

aspects of TMS, including (i) a brief history; (ii) some technical details and (iii) an overview of the neurophysiological principles and mechanisms.

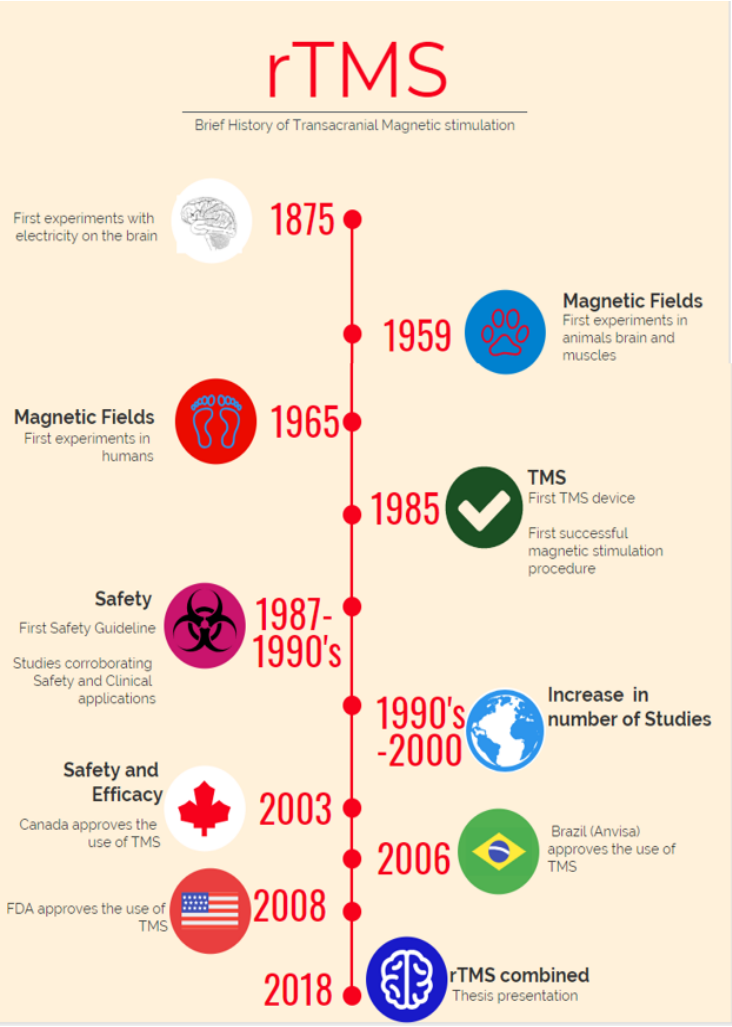


Figure 2: TMS timeline

1.7.1. Brief History of Transcranial Magnetic Stimulation

Neuromodulation techniques were used long before the development of procedures allowing storage of electrical energy and controlled application (Figure 2). About 2,000 years ago, during the Roman empire, the physician and emperor Claudius described the use the "torpedo fish" for the treatment of headaches. This fish belongs to the species of electric rays that are known to release electric pulses (85). Since then, several applications of electrical stimulation for different therapeutic purposes have evolved, such as analgesia, cardiovascular resuscitation and neuromodulation (86).

In the XVIII century, Luigi Galvani, an Italian physician, explored the effects of electrical discharges in animal tissues. During one of his experiments, an assistant touched accidentally a sciatic nerve of a frog with electrically energized metallic object, which produced a muscular contraction (87, 88).

For this reason, Galvani is considered the first "researcher" to explore the relationship between electricity and muscle contraction. His experiments led to the conclusion that the nerve cells and muscle tissue interacted through electrical discharges. In the eighteenth and nineteenth centuries, studies about the relationship between electric charges and cellular activity were largely boosted by the development of the first "voltaic battery" by Alessandro Volta. However, the exploitation

of electrotherapy was leveraged when researchers such as Benjamin Franklin, Leyden Jar, Cavallo, Faraday and Ure Aldine began to explore the electric current and its possible applications in clinical areas (87, 88). In 1874 doctor Roberts Bartholow was the first to perform electrical stimulation in the cerebral cortex in humans using the "voltaic battery".

However, the use of magnetic stimulation began later with the first experiments performed during the late 1900s, since the capacitors were not capable to generate high intensity or fast frequency magnetic fields. Therefore, the current use of electromagnetic induction for transcranial stimulation dates back to 1985 when Baker et al introduced TMS (89).

Based on the principle of electromagnetic induction postulated by Michael Faraday in 1838, TMS consists of a coil through which an electric current flow and induces an electromagnetic field that penetrates the brain. Baker has shown that the electric current generated by a TMS pulse is able to depolarize the neurons, since the placement of the coil on the region near the motor cortex resulted in movements in the contralateral arm (89, 90). Initially, the main interest of the technique was for mechanistic, non-invasive neurophysiological studies. However, the interest to use rTMS as a therapeutic intervention increased rapidly. and the number of publications reached the mark of 160 articles in 1996. Currently it reaches the mark of 3.823 articles on one of the largest scientific databases in the field of medicine and health (Pubmed).

Non-invasive brain stimulation has become a tool for clinical use, mainly after the FDA approval (Food and Drug Administration Administration) in 2008 for the use of rTMS in the treatment of depression. Four years later, in 2012 the Brazilian Federal Council of Medicine (CFM) recognized the clinical use of rTMS in the treatment of depression, auditory hallucinations of schizophrenia and for surgical brain mapping. However, TMS, as well as other NIBS techniques such as tDCS, has been studied for the treatment of several other neurological diseases and psychiatric disorders, including Parkinson's, dementia, neuropathy and stroke (91).

1.7.2. rTMS- technical aspects

TMS uses electromagnetic induction to generate an electrical current in the brain. Fundamentally, a TMS device consists of a small coil (copper wire) connected to an electrical capacitance, that when switched on, allows a large electrical current to flow through the wire. This current produces a magnetic field at orthogonal angles to the coil plane. The equipment

produces voltages of 500 V to 4000 V, for 50 to 100 ms; this corresponds to an energy equivalent of 400 J to 2500 J. This energy is discharged as an electric pulse of great intensity through the coil. The peak of magnetic field strength generated is approximately 1.5 to 2.2 Tesla but may vary based on the parameters of each equipment. These magnetic fields penetrate the brain through the scalp and skull (1.5-2 cm) and are able to induce current fluxes in brain tissue without being attenuated . For more information on the technical aspects of magnetic stimulation please refer to Rossini 2015(92, 93).-

1.7.3. Principles of TMS and mechanism

Recently, the term *neuromodulation* has been used more widely with variable meaning depending on the context. However, according to the International Society of Neuromodulation (ISN), *neuromodulation* is defined as "the use of technological equipment with direct action on the neurons activity" (68). In other words, it consists of a procedure that modulates neuronal activity by electrical or pharmacological agents. This concept will probably undergo modifications and adjustments in order to reflect the rapid and dynamic advances in the area.

Although TMS is applied in specific brain areas, its effects extend to other regions, through the existent connections between the cerebral cortex with other brain areas. In this way, TMS modulates cortical-cortical and cortical-subcortical connections and functional networks of the nervous system.

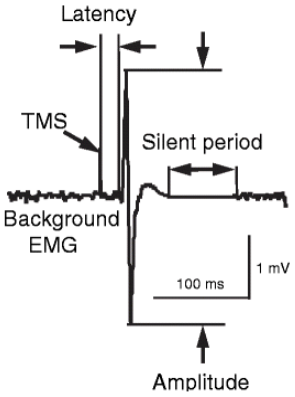
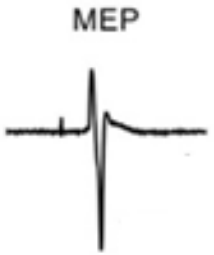
Several parameters can influence the effects TMS; as the same technique can be used in different ways, by changing stimulation parameters such as: magnetic pulse waveform; shape of the coil; stimulation intensity, frequency and pattern of stimulation pulses. To date there are three main types of TMS protocols based on the pattern of pulse delivery: single pulse (TMS); paired pulse (ppTMS); and repetitive pulse rate (rTMS). Each of these types of stimulation has well defined characteristics. In the following section we will briefly discuss these applications.

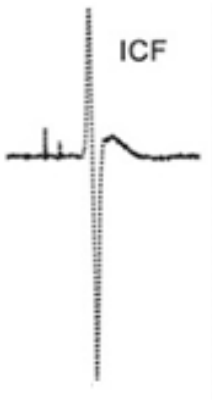
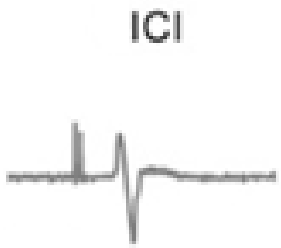
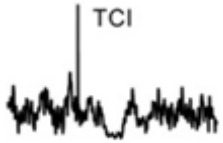
Single pulse TMS and ppTMS are frequently used for diagnosis and to evaluate the effect of an intervention, since motor cortex stimulations can be used to assess the conduction and integrity of the descending cortico-nuclear and cortico-spinal connections. Changes in motor excitability can easily be measured by recording the amplitude of motor evoked potentials (MEP) elicited by a TMS pulse. Several other neurophysiological measures, such as corticomotor threshold (MT),

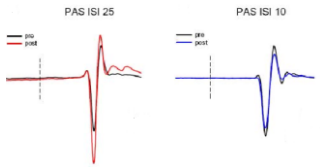
MEP latency, Cortical Silent Period (CSP) duration, Intracortical facilitation (ICF), Intracortical Inhibition (ICI) or Transcallosal inhibition (TCI) can be used to provide knowledge of disease or intervention-related changes in cortical excitability (Table 1)(93).

For all these measurements the muscle contraction generated after the TMS pulse, is captured by surface electromyography (EMG), which is called the motor evoked potential (MEP). The lowest stimulus intensity (magnetic pulse) which evokes a MEP of at least 100 uV peak to peak, in half of ten successive attempts at rest is known as the resting motor threshold (rMT)

Table 1: Summary of single and paired pulse TMS measures

TMS Variable	Definition	Graphic representation	Plasticity changes
Resting Motor threshold (rMT)	Is the minimal intensity of stimulation necessary to elicit a MEP of 100uV in the target muscles, in at least 50 % of the attempts(93).		Changes of rMT might represent an indirect measure of intrinsic plasticity in human motor cortex (93).
Motor evoked potential (MEP)	A MEP is the response recorded in the target muscle after a TMS stimulus (93).		MEPs can be used to evaluate the integrity of cortico-spinal pathways; MEP amplitudes represents changes in the synaptic plasticity (LTP-like and LTD-like plasticity) and therefore changes of MEP amplitudes represent changes in the motor cortex plasticity (93).

Intracortical Facilitation (ICF)	It can be elicited when a subthreshold (80% of rMT) cortical stimulation is followed by a suprathreshold (120% of rMT) stimulus at an intra-stimulus interval (ISI) of 6–30 ms, resulting in increased MEP amplitude (93, 94).		The physiological basis of ICF are still poorly understood (95, 96). It is suggested that this form of facilitation involves glutamatergic circuits in M1(97). The modulation of intracortical excitability can induce plasticity; however, most of rTMS trials do not report significant changes in ICF.
Intracortical Inhibition (ICI/SICI)	It can be elicited when a subthreshold (80% of rMT) cortical stimulation is followed by a suprathreshold (120% of rMT) stimuli at an ISI of 1–6 ms, resulting in a decreased MEP amplitude(93, 94).		ICI can reflect the balance between inhibitory and excitatory networks in the motor cortex. It is believed to be related to neuronal refractoriness and post synaptic inhibition mediated by GABA receptors (93).
Transcallosal Inhibition (TCI)	It can be elicited by cortical stimulation of M1 of one hemisphere followed by suprathreshold (120 or 130%) stimuli over M1 of the contralateral one (93), resulting in decreased MEP amplitude in the initially stimulated hemisphere.		TCI responses are likely produced by interhemispheric excitatory pathways through the corpus callosum and synapses from local inhibitory circuits in the target M1 (10, 98).

Paired associative stimulation (PAS)	This method involves a TMS test stimulus preceded by conditioning electrical stimulation of the median peripheral nerve. It can be facilitatory (25 ms) or inhibitory (10–15 ms) depending on the intervals between the peripheral and cortical stimulus (53, 93, 99).		PAS25 is known to induce LTP-like response affecting primary long latencies intracortical circuits (93).

There are different ppTMS protocols (ICF, ICI and TCI, see Table 1), all consisting of two pulses applied within milliseconds. The intensity of the first pulse is usually 80% of the rMT, being subthreshold, followed by a second pulse suprathreshold, generally at 120% of the rMT. If the interval between stimuli is around 2 to 3 milliseconds, the first stimulus should be able to inhibit the response of the second one, and the amplitude of the MEP generated will be smaller than the MEP generated with the single pulse TMS. When the interval between stimuli is around 10-12 milliseconds, it is expected a MEP with bigger amplitude. However, if the interval is approximately 6 milliseconds the MEP is expected to have the sample amplitude of one elicited by single pulse TMS. In the first case (ICI), the percentage reduction of the MEP is interpreted as an indirect measure of the GABAergic activity of the interneurons and, in the second case (ICF), the percentage increase of MEP would be a measure of glutamatergic activity (93, 100, 101).

In turn, repetitive TMS consists of the application of pulse trains of magnetic fields with the same intensity, at a specific frequency. The rTMS series may exert modulatory effects on cortical excitability and promote both inhibition or facilitation. The results depend on the frequency of the stimulation pulses, since low frequencies of rTMS (≤ 1Hz) generally leads to inhibitory activity (decreasing neuronal excitability), while high-frequency rTMS (> 1Hz), usually promotes excitatory activity. The final effects - excitation or inhibition - arise from the stimulation frequency and are dependent on the basal level of brain activity. Furthermore, the outcome is probably influenced by homeostatic mechanisms regulating brain excitability.

The neurophysiological mechanisms promoted by rTMS are still under investigation. The basic principle of rTMS is the electrical excitation of the axons with the production of action potentials and the release of neurotransmitters in the post-presynaptic cleft. The rTMS stimulation results in the excitation of the neuronal circuits involved with synaptic plasticity. Past studies have shown an increased blood flow in the areas of high frequency rTMS stimulation and it is known that neuronal activity is directly related to local blood flow (25). According to current theories, the long-lasting therapeutic effects of rTMS are related to two phenomena: long-term potentiation (LTP) and long-term depression (LTD; (102). These key mechanism can be induced by the choice of the rTMS parameters: high frequency rTMS or theta bursts stimulation can induce LTP (103). In this type of stimulation activity in the presynaptic neuron is followed by the stimulation of a postsynaptic neuron within several tens of millisecond. On the other hand, low

frequency stimulation can induce LTD, since the stimulation of a postsynaptic neuron that is followed by the stimulation of a presynaptic neuron within several tens of milliseconds. However, these changes in neuronal response are not observed when the time difference is longer than 100 ms (104).

These molecular mechanism were better illustrated in human studies showing that 1 Hz of magnetic stimulation lead to a decrease of induced muscle responses; LTP-like responses (105-109). Moreover, a 15-min low frequency rTMS session at 0.9 Hz (800 pulses) with a stimulation intensity of 115% of the motor threshold results in a 20% decrease in the induced muscle response, that lasted 15-min beyond the end of the stimulation (106).

Besides that, an increase in cortical excitability is observed after high-frequency stimulation of the primary motor cortex (M1). In one of the first rTMS studies, Pascual-Leone and collaborators (110) showed a 50% increase in the induced muscle response after 20 high frequency rTMS pulses at an intensity of 150% the motor threshold.

This theory, of high frequency rTMS resulting in LTP and low frequency rTMS in LTD, is the most accepted one used to explain the mechanism of rTMS. However, this approach has numerous drawbacks (111) and recently it is known that rTMS could have alternative mechanistic effects, by altering other cell process such as changes in gene expression, affecting plasticity of Glial cells and inducing neuroprotective mechanisms. rTMS also has an effect changing the expression levels of different cell receptors and neuromodulators (111, 112). Previous studies in animal models showed that rTMS stimulation can lead to the decrease of β-adrenoreceptors in the frontal and cingulate cortices; an increase in NMDA receptors in the ventromedial thalamus, amygdala and parietal cortex (111, 113); an increase in nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) levels in the cerebral cortex, gyri and hippocampus. However, few observations were made regarding morphologic changes in the neurons after several days of rTMS treatment.

Moreover, there are alternative explanations for the therapeutic effects of TMS, related to the biophysical effects associated with the magnetic field that go beyond the content of this thesis (Figure 3).

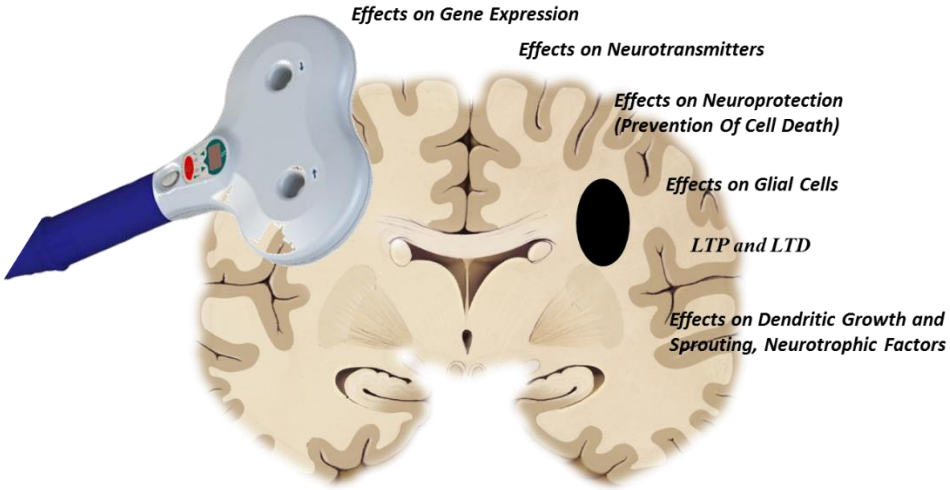


Figure 3: Summary of TMS effects

1.8. Repetitive Magnetic Stimulation improves motor function in stroke subjects

Functional imaging and TMS have been used to better understand changes in cortical plasticity, cortical excitability and reorganization, since these phenomena have been associated with motor rehabilitation after a stroke. TMS can measure changes along the different stages of stroke recovery (acute, subacute or chronic) (114). These changes are maybe associated with neuro-biologic alterations resulting from the injury such as neuronal loss, cerebral blood flow reduction, activation of neuro-protective pathways, alteration of cortical inhibition and membrane excitability and other changes (100, 115, 116).

Single-pulse TMS can be used to assess cortico-spinal integrity and cortical excitability in stroke (117, 118). Stinear and colleagues have shown that the presence or absence of a MEP in the paretic limb can help to predict the response to motor training in patients with chronic stroke (119). Moreover, the measurement of MEPs is part of a multimodal algorithm used to predict functional outcomes in acute stroke patient. Levels of intracortical excitability can be evaluated using paired-pulse TMS paradigms (94, 120) and altered patterns of intracortical inhibition and facilitation in both, the ipsilesional and contralesional hemispheres have been demonstrated in acute and chronic stroke (121, 122). In addition, TMS has been used to assess interhemispheric

interactions in chronic stroke patients (123, 124), showing that alterations in the transcallosal structure and function were associated with upper extremity motor impairment (125).

Aside from characterizing cortical excitability as discussed before, rTMS can be used to modulate brain activity in acute or chronic stroke phases. The use of rTMS in post-stroke recovery relies on the hypothesis that the stimulation could induce plastic change in the brain reverting the maladaptive reorganization induced by a stroke. Recent animal and human studies have demonstrated that cortical brain stimulation with invasive and non-invasive brain stimulation improves motor function in stroke patients (5, 126) (90, 127-130); including clinical trials with repetitive transcranial magnetic stimulation (rTMS).

Currently, different NIBS protocols have been used in post-stroke motor rehabilitation and beneficial effects on motor function can be induced, with either inhibitory 1-Hz rTMS (126) or cathodal-tDCS (131) over the unaffected hemisphere (usually in the chronic phases); or excitatory 3-Hz rTMS (132) or anodal-tDCS of the affected hemisphere in more acute phases. Nevertheless, few studies have been performed applying rTMS in the acute phase of stroke and the current evidence support more its use in the chronic stage. Several researchers have shown motor function improvements suggesting that behavioral changes can be promoted by rTMS even after the critical period with maximal motor recovery potential (91). Previous clinical trials demonstrated that low-frequency TMS may enhance motor performance when applied over the contralesional hemisphere via a potential modulation of inter-hemispheric competition (22).

In a review with 50 studies on noninvasive brain stimulation of the motor cortex to modulate motor function, a significant pooled effect size towards improvement of motor function of active stimulation compared to sham intervention was found (133). However, recent reviews and metanalysis have been challenging the efficacy of rTMS, since in most of the studies the effects were small and short-lived (134).

1.9. Combination of rTMS and Fluoxetine

In this context, the combination of bottom-up strategies such as the use of fluoxetine to promote plasticity and motor recovery in stroke can be potentiated by a top-down approach, such as rTMS (Figure 4).

This synergistic approach has shown positive outcomes in other disorders such as the treatment of major depression. In a study from Brunoni et. al, the combination of transcranial direct current stimulation (tDCS) and sertraline was used in moderately to severely depressed patients and proved to be more effective than each therapy alone, as well as when compared with the placebo intervention (135).

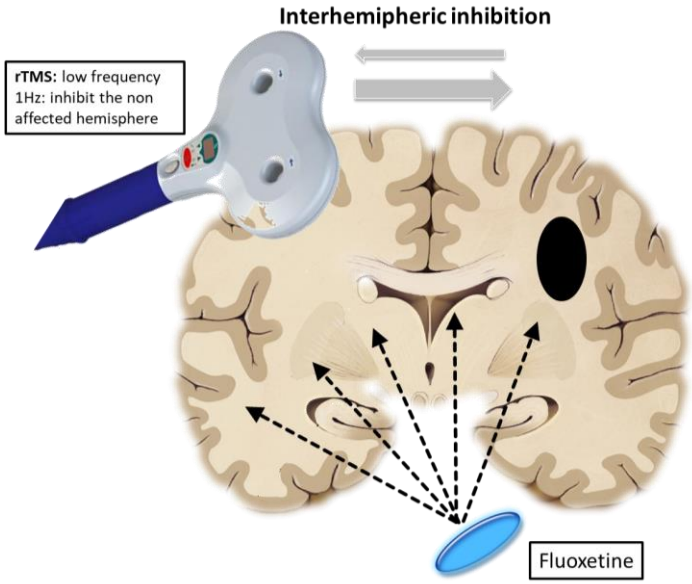


Figure 4: Schematic representation of neuromodulatory mechanisms on motor recovery
rTMS at 1Hz over unaffected M1 will regulate interhemispheric inhibition, while fluoxetine will provide bottom-up regulation through pharmacological effects. Modified from Fregni et al. 2007.

2. HYPOTHESIS AND SPECIFIC AIMS

The objective of this mechanistic trial was to evaluate if the combination of low-frequency rTMS with fluoxetine generates greater benefits over recovery of motor function when compared with pharmacotherapy alone and placebo.

Aim 1: Determine whether low-frequency rTMS of the unaffected M1 associated with fluoxetine offers an additional benefit on motor function recovery over pharmacotherapy (fluoxetine) alone after three months of the combined therapy.

The first aim of the study was to assess the effects of the combined therapy (low-frequency rTMS applied over the primary motor cortex (M1) in the unaffected hemisphere and fluoxetine) on motor function improvements - - when compared to fluoxetine alone and placebo groups.

Our hypothesis is that the combination of rTMS with fluoxetine would exert higher motor gains when compared to the fluoxetine alone and placebo groups. And that both groups (combined treatment and fluoxetine alone) were able to show a superior effect when compared to the placebo only.

Additionally, we evaluated if these effects were dependent on changes in mood. Lastly, we as well assessed the preliminary safety effects of these interventions

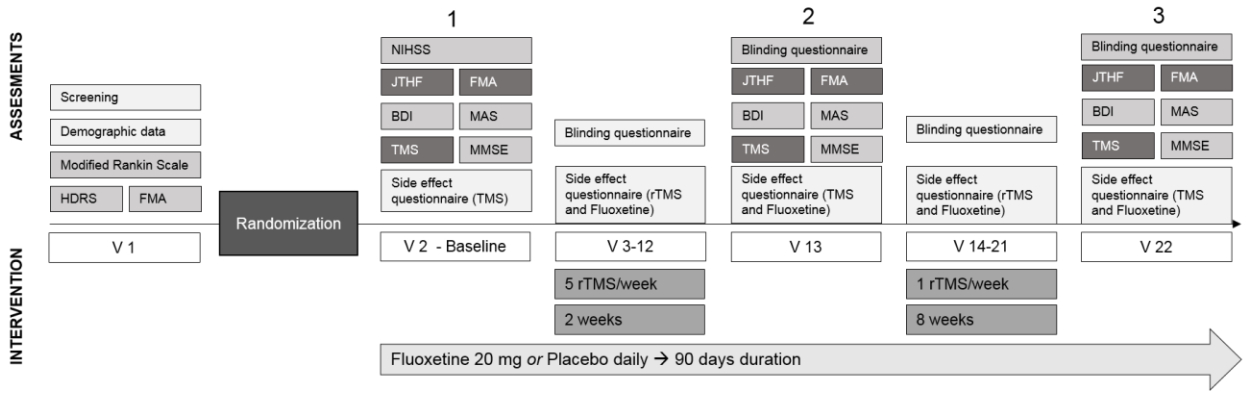
The second aim was to evaluate the mechanisms of the intervention by assessing the neurophysiological changes induced over the primary motor cortex as indexed by motor evoked potentials (MEPs) and cortical excitability/inhibition measurements (secondary aim, is briefly discussed in this thesis since the main aim here is the changes in motor functional outcomes).

4. MATERIAL AND METHODS

4.1. Study design

This exploratory double blinded randomized clinical trial (NCT02208466) was reviewed and approved by Partners Institutional Review Board - Spaulding Rehabilitation Hospital. A written informed consent was obtained from all enrolled participants before all trial procedures. This study was structured in 22 visits, all subjects started the medication (fluoxetine or placebo) at baseline. At three timepoints major assessments were performed; at baseline (1, V2); after two weeks with daily stimulation sessions (total 10 sessions, sham or active rTMS) (2, V13) and at the end of the study after the subjects received weekly stimulation session over another 8 weeks (total 8 sessions, sham or active rTMS) (3, V22). This last assessment visit was performed after 90 days of drug or placebo treatment and after a total of 18 sessions of either sham or active rTMS. Figure 5 shows the study design schema.

Figure 5: Study visits outline



V1: Screening; V2: Baseline assessments (Day 0), study medication given; V3 (2 weeks after V2): daily rTMS sessions begin; V13: Day 30 assessments; V14-21: 8 weekly rTMS sessions; V22: Day 90 assessments. HDRS = Hamilton Depression Rating Scale; FMA = Fugl-Meyer Assessment; JTHF = Jebsen-Taylor Hand Function; BDI = Beck's Depression Inventory; MAS = Modified Ashworth Scale; TMS = Transcranial Magnetic Stimulation; rTMS = repetitive TMS; MMSE = Mini-Mental State Examination.

4.2. Subject selection

Out of 44 enrolled patients with ischemic stroke, 27 were randomized. Participants were considered eligible to participate if they fulfilled the following inclusion criteria and none of the exclusion criteria:

Inclusion Criteria:

- Ischemic infarction within the past 2 years that has caused hemiparesis or hemiplegia, as self-reported and/or confirmed by medical record.
- Older than 18 years.
- Upper extremity weakness defined as a score of >11 and ≤56 on the Fugl-Meyer motor scale.
- Minimal pre-stroke disability defined as a score of <3 in the Modified Rankin Scale.
- Be able to follow instructions and participate in the 2 hours assessment visits with short breaks.
- Be able to provide informed consent.

Exclusion Criteria:

- Any substantial decrease in alertness, language reception, or attention that might interfere with understanding instruction for motor testing.
- Excessive pain in any joint of the paretic extremity (not applicable to severe stroke subjects), as self-reported.
- Contraindications to single pulse TMS such as: history of seizures, unexplained loss of consciousness, any metal implants in the head, frequent or severe headaches or neck pain, any other electronic implanted medical devices such as pacemakers, defibrillators, or implant medication pump.
- Fluoxetine intake in the past five weeks.
- Intake of any other SSRI at the time of enrollment or in the previous month.
- Intake of any other medication, which likely has adverse interaction with fluoxetine (all the medications the patient is taking will be carefully reviewed before the beginning of the trial and monitored during the whole study, as noted below in "Monitoring of important drug interactions").
- Active depression on admission to Spaulding Rehabilitation Hospital (SRH) defined by a score of 24 or higher in the Hamilton Depression Rating Scale (HDRS).
- Concurrent medical condition likely to worsen patient's functional status in the next six months, such as: cancer, terminal heart, kidney or liver disease, as self-reported and/or confirmed by medical record.

- Pregnancy.

4.3. Subject enrollment and randomization

Eligible subjects were identified using different methods of recruitment. A pre-screening form was applied to all potential participants in order to pre-verify the subject's eligibility, this step was in some cases performed by a phone interview or in the case of inpatients from SRH by a brief check of the medical records. The next step consisted in scheduling a screening visit at the Neuromodulation center. In this visit the informed consent was obtained by a licensed physician or the principal investigator (PI), neither of them was involved in the patient's care at SRH. The PI or licensed physician clearly explained all the procedures and risks of the testing outlined in the consent form. The subject received the informed consent at least 24h hours before this visit and were encouraged to study it carefully and prepare questions. If necessary, it was given an hour to consider their decision. The PI or licensed physician and study team answered any questions regarding the study at the time consent was given. Screening process began only after informed consent was signed. If all the inclusion were fulfilled and no exclusion criteria was applied, the participants were screened in the study. Otherwise the study team explained the reason why the subject was not eligible, and the participant could choose whether they would like to be contacted again in the future for a possible re-screening. Once enrolled, the subject was able to pause or terminate his/her participation at any time.

4.4. Intervention Details and Study overview

This trial used three groups to address the research question, group 1: active rTMS+fluoxetine, group 2: sham rTMS+fluoxetine and group 3: sham rTMS+placebo.

4.4.1. Repetitive transcranial magnetic stimulation

Low frequency rTMS stimulation (active stimulation)

During the stimulation session, the participant received a train of low-frequency rTMS stimuli to the primary motor cortex of the unaffected hemisphere.

- The resting motor threshold (MT) of the first dorsal interosseous (FDI) muscle in the unaffected hemispheres was measured. The participants received the rTMS over the

area corresponding to the "hot spot" for stimulation as defined by motor threshold determination, as described by prior rTMS application studies (92, 93).

- Low frequency rTMS stimulation was performed, with an intensity of 100% of rMT and frequency of 1Hz. In total 1200 pulses in a single, continuous train lasting 20 minutes were applied.

Sham rTMS stimulation:

- For sham rTMS stimulation, the coil was placed at the same location used for the active stimulation. However, we replaced the active coil with a sham coil that was able to provide auditory cues mimicking the stimulation (same frequency and equal train duration), but without providing active stimulation.

The subjects randomized to receive sham rTMS had the opportunity to enroll into an open label phase (results of the open label phase are in section 5.10) at the end of their participation in the randomized portion of the trial. This consisted of 10 daily active stimulation sessions that were carried out over the course of two weeks. Subjects only received rTMS as the study staff was not able to provide fluoxetine.

4.4.2. Drug Intervention

Fluoxetine:

- Subjects received 20 mg fluoxetine for the first time at their baseline visit. They took the study drug by mouth once daily from this day until the protocol was completed (for 90 days). This was the same regimen as in the FLAME study (28).

Placebo control:

- The placebo pill had same appearance of the real drug. The treatment protocol was kept the same for both groups. Only the pharmacist and a study staff not involved in the protocol were aware of drug randomization. The procedure for taking the study drug and the monitoring of compliance was the same for the patients receiving the active drug.

4.5. Monitoring of important drug interactions

Participants were monitored for any medication that they were taking that could potentially cause interactions with fluoxetine. Since fluoxetine is a potent inhibitor of CYP2D6 and CYP3A4, drugs metabolized by these enzymes were avoided or their dosage was modified. Furthermore, any drugs that increased the risk of serotonin syndrome or QT- interval prolongation were as well avoided as fluoxetine inhibits serotonin re-uptake.

Prior to patient's enrollment, the licensed physicians collaborating with the study – Dr. Black-Shaffer - performed a close review of the medications and determined if any drug the patient was taking had any possible interactions with fluoxetine. If needed and if possible, dose adjustments of these medications were made. A log of the medications that the patients were taking, including details about the dosage was kept throughout the trial and patients were asked to inform the study staff if any changes were made. Study staff confirmed with subjects weekly that no changes were made.

4.6. Clinical Assessments

A rater blinded to the treatment arm was performing the following assessments:

1. Jebsen Taylor Hand Function Test (JTHF): This was our primary outcome. This test was designed as a broad measure of hand function in activities of daily living. It provides information on the time required to turn cards, pick up small objects, simulate feeding by picking up beans with a spoon, stack checkers, and lift an empty and a 500g full can This instrument showed to be sensitive to measure motor changes induced by motor cortex stimulation. The maximum time to execute each task is 120 seconds. If the subjects were not able to complete one of the tasks, a score of 120s was given. For the analysis, the writing part was excluded - considering difference in stroke side regarding the dominant hand, and therefore the maximum score in our trial was 720 seconds.

2. Fugl-Meyer motor scale (FMMS): This instrument was the main outcome used in the FLAME study and is widely used for assessment of motor recovery after stroke.

3. Modified Ashworth Scale: This instrument is a 6-point rating scale that is used to measure muscle tone. This test is performed by moving the body part through the joint

and assess the range of motion (ROM), with no specification about the speed of the movement.

4. Beck Depression Inventory (BDI): This 21-item multiple-choice test measures the presence of, and the degree of depression in adults.

5. Mini Mental State Examination (MMSE): The MMSE is a brief screening instrument used to assess cognitive abilities. Consistency of MMSE scores suggest that a subject had no cognitive changes throughout the intervention period that may have affected test performance or carryover of the program.

6. National Institute of Health Stroke Scale (NIHSS): is a tool used to objectively quantify the impairment caused by a stroke. It is composed of 11 items, each of which scores a specific ability between a 0 and 4.

7. Visual analogue scale (VAS) for anxiety: This tool is a visual scale of 0-10 where the subject can rate their level of anxiety where 0 is no anxiety, and 10 is the worst anxiety that the subject has ever felt.

8. Visual Analogue Scale (VAS) for pain: This tool is a visual scale of 0-10 in which the subject can rate their level of pain where 0 is no pain, and 10 is the worst pain that the subject has ever felt.

9. Side Effects / Adverse event tracking Questionnaire for rTMS: After each session, subjects completed a questionnaire to evaluate potential common adverse effects of rTMS (headache, neck pain, itching and redness at the site of stimulation) on a 5-point scale, where 0 was not present and 5 was severe.

10. The Antidepressant Side-Effect Checklist (ASEC): The ASEC is a questionnaire that assesses the possible appearance of side effects related to the use of common antidepressants, their severity and if they are linked or not to the drug.

11. Blinding Questionnaire: This questionnaire was performed at the end of the daily stimulation sessions (V13) and at the end of the weekly stimulation sessions (V22). This questionnaire asked the subjects whether the stimulation was active or sham rTMS. The

confidence of these responses was rated from 0 to 5 – 0 being not confident and 5 totally confident-. If the subject was interested in knowing what stimulation he/she received, the co-investigator informed the subject when his/her participation in the trial ended.

12. Medication Diary: A log of the hours of therapy that patients received and the medications they were taking was updated every week and kept during the entire participation in the trial.

4.7. Assessment of cortical excitability and plasticity

A rater blinded to the treatment performed the TMS assessments to determine whether low-frequency rTMS of the unaffected M1 associated with fluoxetine offered an additional benefit on motor function over pharmacotherapy only.

We investigated changes in cortical excitability by evaluating the motor evoked potential (MEP) and the resting motor threshold (MT). We also measured intracortical excitability using the technique of paired-pulse, and interhemispheric differences using transcallosal inhibition of both, the affected and unaffected M1.

The TMS assessments were performed with a Bistim^2 stimulator (Magstim Company LTDA, UK) and a commercially available 70 mm figure-of-eight coil. Responses to stimuli applied to the motor cortex were recorded from the contralateral first dorsal interosseous (FDI) muscle. To record MEPs, silver/silver chloride electrodes were placed over the muscle belly (active electrode) and joint or tendon of the muscle (reference electrode). A third electrode as a ground, was placed over the wrist. MEPs were amplified and filtered using a Powerlab 4/30 with a band pass filter of 20-2000 kHz. Signals were fed to a personal computer for off-line analysis using data collection. We investigated the resting motor threshold measured following the protocol described by Rossini et al. (92, 93)(Figure 6).

I. To study the MEPs, we used 120% of the MT in the first dorsal interosseous muscle. Stimulation intensity was kept constant for each subject. The MEPs were recorded and stored in a computer for off-line analysis. We recorded 10 MEPs for each time point and averaged their peak-to-peak amplitude, as well as their area-under-the-curve.

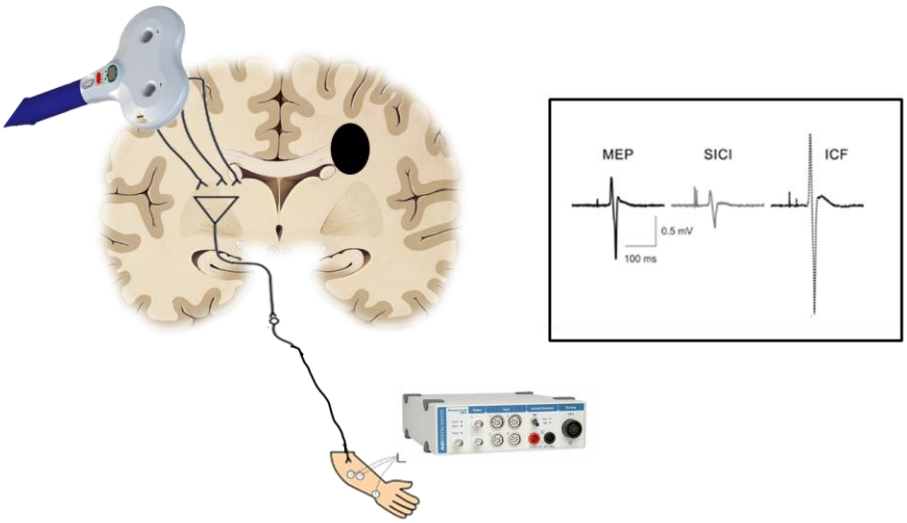


Figure 6: Schematic representation of single and paired pulse stimulation
Assessments performed during this study to measure cortical excitability

II. For the paired-pulse study, a subthreshold conditioning stimulus (80% of MT) was applied, followed by a second (suprathreshold) test stimulus (120% of the MT) after a variable interstimulus interval (ISI). We used the following ISIs: 2, 3, 6, 10, and 12 ms.

III. To measure changes in transcallosal inhibition, a suprathreshold stimulus (130% of the MT) was applied to the motor cortex of one hemisphere, and ten milliseconds later a second suprathreshold stimulus was applied to the contralateral motor cortex.

IV. To measure LTP-like effects in the motor cortex, we used the measure of paired associative stimulation (PAS). For PAS, 160 pairs of peripheral nerve stimulation, followed by a TMS stimulus of motor cortex were delivered at a frequency of 0.1 Hz. Stimulation of the median nerve preceded TMS by 25 milliseconds (PAS-25). It has been shown that in PAS-25, the two inputs via peripheral nerve stimulation and via TMS reaches motor cortex simultaneously which results in facilitation of the MEP induced by TMS (ref Stefan, 2000). PAS-25 has been used as a robust marker to assess cortical plasticity in M1.

Table 2: Study Visit Summary

Assessments and Interventions	Screening	Visit 1	Visit 2-11	Visit 12	Visit 13-20	Visit 21
Review of eligibility criteria	X					
Demographic Data	X					
NIHSS		X				
Modified Rankin Scale	X					
HAM-D	X					
Jebsen Taylor Hand Function Test (JTHF)		X		X		X
Visual analogue scale (VAS) for anxiety		X		X		X
Modified Ashworth Scale		X		X		X
Visual Analogue Scale (VAS) for pain/comfort		X		X		X
Mini Mental State Examination (MMSE)		X				X
Fugl Meyer (FM)	X	X		X		X
Beck Depression Inventory (BDI)		X		X		X
TMS Assessments		X		X		X
STIMULATION 1. Low-Frequency rTMS + fluoxetine **OR** 2. Low-Frequency sham rTMS + fluoxetine **OR** 3. Low-Frequency sham rTMS + placebo			X		X	
Side Effects Questionnaire for rTMS (post-stimulation) and for fluoxetine		X	X	X	X	X
Blinding Questionnaire (post-stimulation)				X		X
Approximate Time	***45 mins***	***3 hrs***	***30 min***	***3 hrs***	***30 min***	***3hrs***

4.8. Blinding Procedures

Participants, family members, treating staff, physicians, data collectors, SRH pharmacists, site investigators were blinded to group assignment. Johnson Compounding and Wellness Center, which was responsible for manufacturing and delivering the correctly randomized study drug, was unblinded.

4.8.1. Guidelines and Procedures for Breaking the Blind

In some cases, it might be necessary to break the blinding to facilitate management of a serious adverse event (SAE). Below is the guideline used tin this trial in case it was necessary to break the blinding:

In the majority of cases where adverse events are noted, however, the decision about whether or not to continue the study drug, and what treatment to provide, if any, can be made without knowing the interventional group. Just as in clinical practice, if an adverse event is thought to be due to a given drug, and that drug is stopped, clearance of the adverse event tends to support the causal connection. On the other hand, if there was a no symptom resolution after stopping the drug the physician was required to search for alternative causes. Thus, in general, the treating physician was in charge of deciding whether or not an adverse event was likely related to the study drug in a blinded fashion, and stop the drug where appropriate, at least temporarily. If the adverse event did not resolve, the physician had to identify another etiology and make a decision, in regard of the study drug in the future.

In order to meet criteria for unblinding the following scenario was expected. In uncommon instances, the treating physician felt that was critical to know which drug the subject was receiving, because:

1. The adverse event was serious; and
2. it was potentially harmful or costly to stop the study drug *and* act simultaneously on other possible causes of the adverse event; and
3. it was dangerous to stop the study drug and wait for a few days to see whether the adverse event resolved before acting on other possible causes.

In all cases, the treatment unblinding form, prompted the PI to address the need to unblind and to ensure unblinding was indeed necessary, this step had to be completed prior to unblinding the

participant. Unmasking/unblinding was considered a serious action. The treating physician was allowed to unblind the participant, without the treatment unblinding form, if unblinding was considered to be essential for immediate clinical management.

The following questions were answered on the treatment unblinding form by the physician in charge whenever unblinding was either considered or implemented:

1. What was the adverse events that lead you to want to be unblinded to the treatment condition?
2. What prevented you from addressing all possible causes of the adverse event simultaneously?
3. What prevented you from stopping the study drug blindly and waited a few days to evaluate the course of the adverse event, and then make decisions about other interventions accordingly?

In all cases in which unblinding occurred, the study physician treating the participant was unblinded and recorded and maintained this data in a confidential log, so the case was reviewed and the reasons for unblinding tracked. The treating physician was reminded not to reveal the treatment assignment to any other staff members unless this information was essential to patient management, or to the patient or the patient's family.

Note: To avoid inadvertent or non-essential episodes of unblinding, the PI (or designee) assured that the covering physician staff, residents, physician's assistants and nursing staff were informed that stringent guidelines were to be followed when starting and stopping all medications. Unblinding was not allowed to occur unless the Guidelines for Unblinding were reviewed and completed by the PI.

A record of each subjects' assignment from Johnson Compounding and Wellness Center, "Active" or "Placebo," was placed in a sealed envelope labeled with the Study Name and ID along with the subject's name and study ID number on the outside. This envelope was placed in a larger envelope labeled with the study name and study ID in the SRH Night Pharmacy Omnicell automated dispensing machine. Only SRH Nursing Managers and the SRH IND Pharmacist, using the Omnicell machine were able to access the study envelope using the product identifier "unblinding key".

4.9. Biostatistical analysis

For the statistical analysis, a descriptive analysis of the data was performed for the demographics and baseline characteristics of the groups using central tendency and dispersion measures (mean and standard deviation for continuous and frequency for categorical variables).

4.9.1.Sample size justification

We planned a sample size based on the SELECT-tDCS trial combining non-invasive brain stimulation and sertraline for the treatment of depression (ref) and the FLAME trial testing fluoxetine on motor function in stroke. Using these two studies, our calculation showed that a sample size of 30 participants was able provide at least 80% power (alpha=0.05) to detect an effect size of 0.9 (considering the treatment difference between the two independent groups) in our main outcome between the active and sham rTMS group + fluoxetine. Although this sample was small and probably relied on large effects found in these two studies, this was a realistic sample for this initial phase II study as the effect size of 0.9 is smaller than the effect size of these two studies. Finally, there was also good statistical power to detect moderate effects of treatment on the secondary outcomes. Given that our subaim 1 (added in the specific aims part) had the goal of assessing whether the effects found in the main comparison were also superior to spontaneous recovery, we added another group of the same size (15 subjects) to provide the same power for the comparison in subaim 1 (active rTMS+fluoxetine vs. sham rTMS+placebo and sham rTMS+fluoxetine vs. sham rTMS+placebo).

All analyses were conducted according to the principle of intention-to-treat, using regression-based single imputation method. We also performed an additional sensitivity analysis in which we used the method of last observation carried forward.

4.10. Primary and secondary outcomes analysis

The primary outcome was the change in motor function as indexed by Jebsen Taylor Hand Function test (JTHF) and the secondary outcome was the change in the Fugl-Meyer motor scale (FMA), both from baseline to the end of the treatment (90 days). Data distribution was assessed through histogram and Shapiro-Wilk test. The differences between groups were investigated using Analysis of Variance (ANOVA) or linear regression models when the data was normally distributed and Kruskal-Wallis test, when not-normally distributed. Also, for categorical

variables, Fisher's exact test were applied. Mean and standard deviation were used to represent normally distributed data, while median and range to represent skewed distribution. For JTHF and FMA, adjusted means were compared and regression models were performed to account for the effects of covariates. All analysis performed were based on two-tailed tests and we accepted a significance level of 0.05.

To investigate the blinding, the Cohen's kappa coefficient was applied (the lower the kappa coefficient value, the higher quality of blinding was achieved). The frequency of adverse events was assessed based on the number of times that the event was reported along the trial.

5. RESULTS

5.1. Recruitment and enrollment procedures

Between June 3rd, 2015, and March 5th, 2018, 279 stroke survivors were pre-screened for eligibility, of which only 44 were potentially eligible and signed the consent from. During the screening process, 17 subjects were excluded because they did not meet the inclusion criteria or due to loss of interest. The main reasons were upper limb motor function score of < 11 on the Fugl-Meyer Assessment (FMA) motor scale (n=14), followed by subjects who did not hold their SSRI prior to baseline (n=2) and 27 were eligible and were randomized. Figure 7 summarizes the enrollment process, group allocation and analysis plan of the trial.

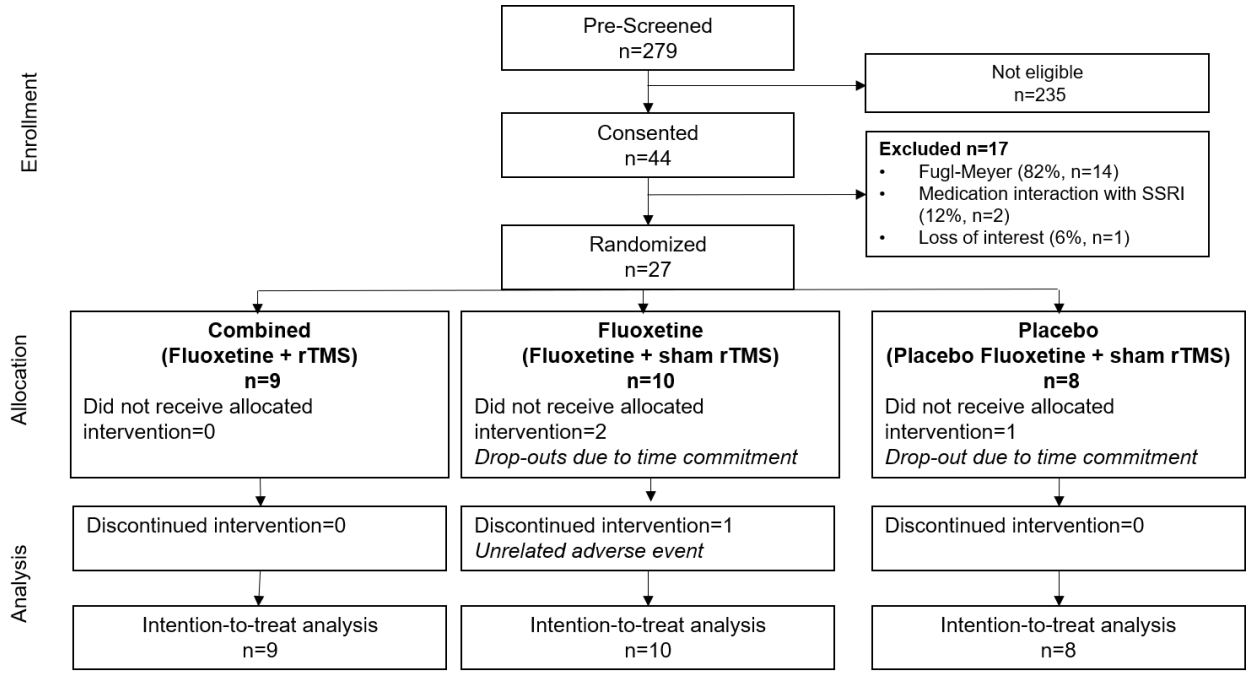


Figure 7: Study flow chart.

The 27 eligible subjects were randomized into one of three possible groups: Combined (Fluoxetine + rTMS), Fluoxetine (Fluoxetine + sham rTMS) and Placebo (Placebo + sham rTMS). However, 4 subjects discontinued their participation in the trial. Two dues to an adverse event, and one decide not to participate after couple of rTMS sessions due to lack of time and one 82-year old subject who thought it was too burdensome to continue.

5.1.1. Recruitment strategies developed for the project

We used broad recruitment strategies and prescreened about 279 potential participants; 44% of those had responded to online advertisements (Google Ads), 15% were referred by their physicians (who were collaborating with the project), 15% were personally approached by our team at stroke and brain injury clinics (Spaulding Rehabilitation Center and Massachusetts General Hospital), 8% heard about the study in other outpatient clinics, 8% responded to our broadly distributed study fliers, 8% were identified by searching databases of medical records, and 4% were referred by other physicians (not collaborating with the project) (Figure 8).

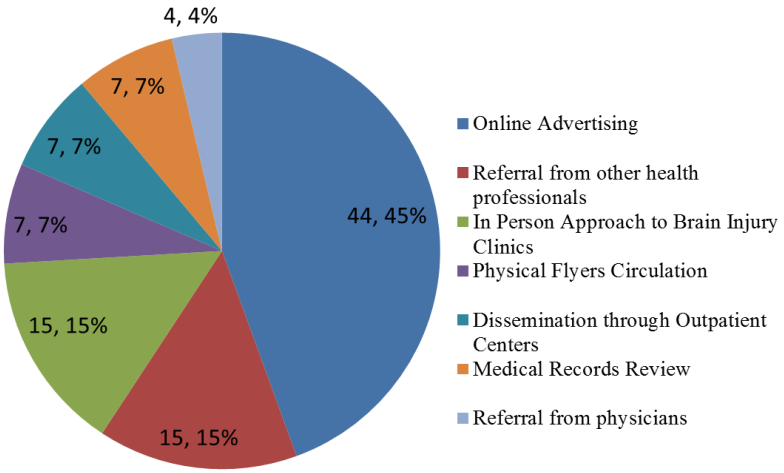


Figure 8: Recruitment Strategies.
Recruitment methods that successfully led to randomization (by percentage of subjects randomized).

The general randomization yield of the study (proportion of randomized participants among potentially eligible subjects by prescreening) was approximately 9.3 % (27 of 279 subjects) and the overall conversion ratio was 10:1, i.e. it was necessary to identify 10 participants to randomize one. Overall, referrals from collaborating physicians had the highest randomization yield at 66%; i.e. patients who were referred by these physicians were the most likely to get randomized. Medical records review had the lowest yield at 1%, despite being a very time-consuming process (eligible patients were often not interested). For more details on the randomization yield of different recruitment strategies, please see Table 3.

Considering the challenges of recruiting this population, particularly as one of our exclusion criteria was fluoxetine use - which is now commonly prescribed after stroke - we had to expand into several different recruitment strategies to reach our target sample (136). We innovated a cost benefit index in an effort to maximize our yield while reducing the cost of recruitment, primarily in terms of human resources. Our strategies and cost benefit index were so effective that we now use them on other research projects at the Neuromodulation Center.

Table 3: Randomization yield in average by each recruitment method.

Recruitment Strategy	**Recruitment Yield**
Referral from other health professionals	66 %
Physical Flyers Circulation	33 %
Referral from physicians	33 %
Dissemination through Outpatient Centers	29 %
In Person Approach to Brain Injury Clinics	22 %
Online Advertising	21 %
Medical Records Review	1 %
Targeted Campaign in Support Groups	0 %
Newspaper Advertising	0 %
Total	9 %

5.2. Demographic and Baseline Characteristics

Most participants were in their 50s, male, Caucasian, overweight and had strokes affecting the right side predominantly. There were no significant differences between the groups

Table 4: Summarizes patient's demographics and stroke characteristics.

	Combined (n=9)		**Fluoxetine (n=10)**		**Placebo (n=8)**	
Age (Years), Mean (SD)	57.22	(9.39)	50.5	(16.57)	57.38	(9.96)
Female, N (%)	4	(44%)	5	(50%)	2	(25%)
BMI (Kg/m^2), Mean (SD)	26.59	(5.71)	26.79	(5.4192)	29.405	(9.36)
White/Caucasian, N (%)	8	(89%)	9	(90%)	5	(63%)
Dominant Hand (Right), N (%)	9	(100%)	8	(80%)	8	(100%)
Affected Side (Right), N (%)	6	(67%)	5	(50%)	5	(63%)
Stroke Location						
RMCA, N (%)	4	(44%)	4	(40%)	1	(13%)
LMCA, N (%)	2	(22%)	5	(50%)	1	(13%)
RPCA, N (%)	0	(0%)	0	(0%)	3	(38%)
LPCA, N (%)	0	(0%)	0	(0%)	1	(13%)
LICA, N (%)	0	(0%)	2	(20%)	1	(13%)
RAChA, N (%)	1	(11%)	0	(0%)	0	(0%)
LAChA, N (%)	1	(11%)	0	(0%)	0	(0%)
Unknown, N (%)	2	(22%)	0	(0%)	1	(13%)
NIHSS, Mean (SD)	5.11	(3.72)	4.89	(2.37)	4.29	(2.43)

	Combined (n=9)		Fluoxetine (n=10)		Placebo (n=8)	
FMA, Mean (SD)	27	(14.89)		(11.67)	25.67	(14.68)
JTHF (s), Mean (SD)	526.91	(279.92)	594.06	(195.28)	439.39	(306.43)
MMSE, Mean (SD)	29.11	(1.36)	29.38	(1.41)	29	(1.2)
HDRS, Mean (SD)	5.77	(3.7)	4.3	(3.02)	6.75	(3.81)
BDI, Median (Min-Max)	7	(0 - 30)	4	(0 - 12)	11	(3 - 14)
Time Since Stroke (Days), Mean (SD)	355.66	(229.51)	339.7	(264.440)	178.87	(225.57)
Total Pills, Mean (SD)	90	(0.00)	89.5	(1.07)	89.5	(1.22)
TMS Sessions, Mean (SD)[1]	17.89	(0.33)	16.89	(2.47)	16.17	(2.85)
MEP Unaffected (mV), Mean (SD)	1.145	(0.68)	1.86	(1.38)	1.52	(0.90)
PT (h/week) Time, Mean (SD)	0.64	(0.76)	0.6	(0.55)	1.23	(1.21)
OT (h/week) Time, Mean (SD)	0.88	(0.67)	0.95	(0.68)	1.44	(1.33)
HE (h/week) Time, Mean (SD)	4.16	(3.4)	6.61	(3.66)	7.04	(4.61)

Data are mean (SD) or number (%). BMI = body mass index; RMCA = Right Middle Cerebral Artery; LMCA = Left Middle Cerebral Artery; RPCA = Right Posterior Cerebral Artery; LPCA = Left Posterior Cerebral Artery; LICA = Left Internal Carotid Artery; RAChA = Right Anterior Choroidal Artery; LAChA = Left Anterior Choroidal Artery; NIHSS = National Institutes of Health Stroke Scale; FMA = Fugl-Meyer Assessment scale; JTHF = Jebsen-Taylor Hand Function Test; MMSE = Mini-Mental State Examination; HDRS = Hamilton Depression Rating Scale; BDI = Beck's Depression Inventory; MEPs = motor evoked potentials; PT = physical therapy; OT = occupational therapy; HE = home exercise.[1]Excluded 3 subjects (2 subjects from placebo group and 1 subject from fluoxetine group) that dropped out before receiving any stimulation sessions.

Even though there were no significant differences between the three groups, some demographic characteristics varied between groups. The average age was slightly lower in the Fluoxetine group (51 years); time since stroke was lower in the Placebo group (179 days); average hours of exercise were higher in the Placebo group (PT, OT and home exercise) and the percentage of patients with MCA stroke was lower in the Placebo group. Additionally, Fugl-Meyer Assessment (FMA) and Jebsen Taylor Hand Function Test (JTHF) scores were higher in the Placebo group (Table 4). All covariates with apparently heterogeneous baseline values among groups were tested in the initial model to assess for any potential confounding effects, which we discuss further in Section 4.4 below.

There were no mean score differences among groups in the NIHSS, Hamilton Depression Rating Scale, Beck Depression Inventory and Mini-Mental State Examination (Table 4). Treatment compliance regarding fluoxetine intake was similar in the three groups. The mean cumulative dose at Day 90 was 90 tablets (±0) in the Combined group, 89.5 (±1.1) in the Fluoxetine group and 89.5 (±1.2) in the Placebo group. Likewise, treatment compliance regarding the number of TMS sessions (18 total) differed only slightly and no significantly between groups. The mean cumulative dose at Day 90 was 17.9 sessions (±0.3) in the Combined group, 16.9 (±2.5) in the Fluoxetine group and 16.2 (±2.9) in the Placebo group, excluding two patients who dropped out and did not receive any stimulation sessions.

5.3. Assessment of Motor Recovery after Stroke

To analyze motor function changes, we first tested all possible covariates described above in a univariate linear regression model. Covariates that achieved a p-value < 0.2 were added in the multivariable model; however, the only covariates that significantly altered the final model were time since stroke (categorized as < or > than 180 days) and the interaction term (time*treatment). In Figure 9 we present the effect of days after stroke on raw motor function changes assessed by FMA and JTHF test (in seconds) in all 3 groups. We presented data from complete case analyses rather than intention-to-treat (ITT) for a clearer visual effect. ITT analyses will be discussed below.

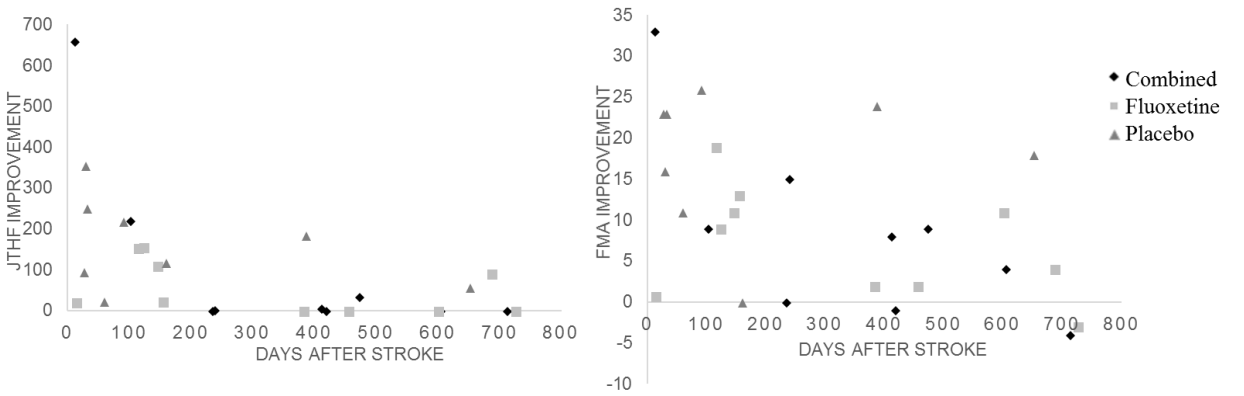


Figure 9: Motor function changes as measured by JTHF and FMA vs. days after stroke (complete case analysis).
FMA = Fugl-Meyer Assessment scale; JTHF = Jebsen-Taylor Hand Function Test.

We added time since stroke and the interaction term (time*treatment) as those were the only covariates significantly affecting the model. However, due to the small sample size we cannot exclude the possibility that other covariates such as age, exercise hours, stroke location and baseline motor function (FMA and JTHF) might have influenced the model but were underpowered. Adding too many covariates would have made the model unstable and unreliable. Note that although all the results for motor function are from the same mixed linear model, we begin with the Combined group (Section 4.5) and then the Fluoxetine group (Section 4.6) for clarity.

Table 5: Motor assessments

		Combined (n=9)	Fluoxetine (n=10)	Placebo (n=8)			
Day 0 to Day 90							
JTHF	Mean (SD)	-102.00 (220.49)	-51.23 (68.85)	162.03 (110.81)			
	Adjusted mean (95% CI)	-214.33 (-289.32, -139.35)	-50.16 (-110.56, 10.24)	117.98 (-197.39, -38.58)	P value, F value	**0.0001**	F (5,21)=9
					P value, 1 vs 2	**0.000**	
					P value, 1 vs 3	**0.005**	
					P value, 2 vs 3	**0.038**	
FMA	Mean (SD)	8.11 (11.07)	6.87 (6.77)	17.62 (8.66)			
	Adjusted mean (95% CI)	10.10 (4.06, 16.14)	6.73 (1.28, 12.17)	15.55 (9.16, 21.95)	P value, F value	**0.0137**	F (3,23)=4
					P value, 1 vs 2	0.401	
					P value, 1 vs 3	0.233	
					P value, 2 vs 3	**0.039**	
Day 0 to Day 30							
JTHF	Mean (SD)	-23.33 (55.45)	-22.03 (44.21)	-21.58 (57.74)			
	Adjusted mean (95% CI)	-17.72 (-60.31, 24.87)	-21.58 (-55.89, 12.72)	-33.15 (-78.25, 11.96)	P value, F value	0.47	F(5,21)=1
					P value, 1 vs 2	0.53	
					P value, 1 vs 3	0.17	
					P value, 2 vs 3	0.31	
FMA	Mean (SD)	5.44 (6.98)	2.67 (3.44)	8.53 (5.96)			
	Adjusted mean (95% CI)	6.22 (2.22, 10.21)	2.61 (-0.98, 6.21)	7.73 (3.50, 11.96)	P value, F value	0.11	F(3,23)=2.2
					P value, 1 vs 2	0.18	
					P value, 1 vs 3	0.61	
					P value, 2 vs 3	0.07	

All JTHF scores are in seconds. Mean was adjusted for time since stroke (categorized with <180 days or >180 days) with a linear regression model for both FMA and JTHF scores. FMA = Fugl-Meyer Assessment scale; JTHF = Jebsen-Taylor Hand Function Test; 1 vs. 2 = combined group compared to fluoxetine group; 1 vs. 3 = combined group compared to placebo group; 2 vs. 3 = fluoxetine group compared to placebo group. Bolded values denote significance at $p<0.05$ and $p<0.001$

5.3.1. Motor function changes in the Combined group

A linear repeated-measures model was performed in which the dependent variable was motor function change (change in FMA or JTHF scores) and the covariates were group (Combined; Fluoxetine and Placebo), time since stroke (categorized as < or > than 180 days) and the interaction term (time*group) (Table 5).

After adjusting for time since stroke, mean improvements (differences between Day 90 and Day 0) in JTHF score were significantly higher in the Combined group (-214.33 s) than in Fluoxetine (-50.16 s, $p<0.001$) and Placebo (-117.98 s, $p=0.005$).

On the other hand, there was no significant difference in mean FMA change for the Combined group when compared to each of the Placebo and Fluoxetine groups.

Moreover, at 30 days, there were no differences in FMA and JTHF scores between the three groups after adjusting for time since stroke.

5.3.2. Effects of Fluoxetine on motor function

After adjusting for time since stroke, the mean JTHF score improvement at the final endpoint (Day 90) was significantly lower in Fluoxetine compared to the Placebo group (-50.16 s vs. -117.98 s, $p=0.038$). In the same way, the mean FMA score improvement was significantly lower in the Fluoxetine group compared to the Placebo group (6.73 vs. 15.55 points, $p=0.039$). At 30 days, there were no differences between the 3 groups (Table 5).

5.4. Effects of the intervention on chronic versus subacute stroke

As time since stroke was a confounder we adjusted the analysis for this variable. In Table 6 we show the differences in JTHF and FMA scores when comparing the “subacute” and chronic stroke populations (categorized into < 180 and > 180 days respectively). In this exploratory analysis, the Fluoxetine group had greater motor improvement in the subacute compared to the chronic stroke populations. Meanwhile, Placebo had greater yet improvement in subacute stroke compared to Fluoxetine. The subacute group did better than the chronic group in both Fluoxetine and Placebo, probably as a result of spontaneous recovery. However, in the Combined group the motor change results were scale dependent: the subacute group showed better improvement on

FMA compared to the chronic stroke group, but the chronic stroke group showed better improvement on JTHF.

Table 6: Mean difference of JTHF and FMA score changes in subacute vs. chronic stroke

		Combined (n=9)	**Fluoxetine (n=10)**	**Placebo (n=8)**
Changes from Day 0 to Day 90				
JTHF	*< 180 days (subacute)*	-6.50	-34.06	-66.69
	> 180 days (chronic)	-28.14	-8.33	-2.00
FMA	*< 180 days (subacute)*	21.00	8.99	18.66
	> 180 days (chronic)	4.43	3.95	14.50

Mean difference for subacute and chronic stroke categories (time since stroke <180 days and >180 days respectively) following the linear regression model for both FMA and JTHF scores. FMA = Fugl-Meyer Assessment scale; JTHF = Jebsen-Taylor Hand Function Test.

5.5. Results of JTHF versus FMA

After adjusting for time since stroke the Combined group did better than the other groups on JTHF, but there were no FMA scale differences between groups. As JTHF assesses mainly wrist and hand function, we correlated it to each of the FMA wrist and hand subscale score and the full FMA score. However, correlating JTHF to the wrist/hand subscale was no different from correlating it to the full FMA scale (Figure 10). Moreover, it is important to point out that most participants had no baseline distal upper limb function, which may have contributed to a floor effect on the JTHF test. Finally, please note that the JTHF test did not include the handwriting assessment (as is common in such studies when handedness and stroke side and location might vary.

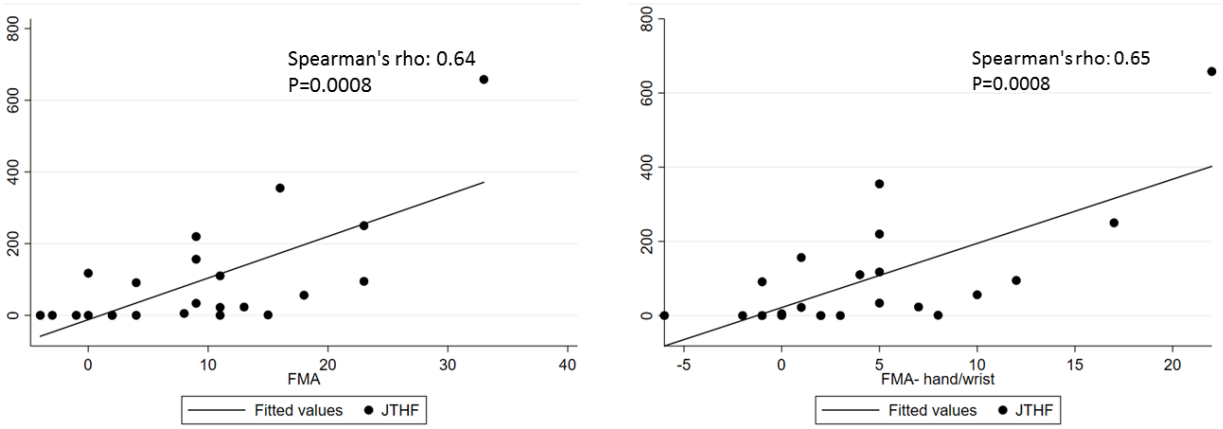


Figure 10: Comparison of motor improvements in JTHF vs. FMA.
The correlation between JTHF and FMA scale (Difference: 90 days – 0 days) was no different from the correlation between JTHF and FMA hand and wrist subscale (Difference: 90 days – 0 days). FMA = Fugl-Meyer Assessment scale; JTHF = Jebsen-Taylor Hand Function Test.

5.6. Motor improvement independent of mood changes

BDI scores did not differ significantly between groups at inclusion, at 30 or at 90 days (Kruskal-Wallis test). Moreover, while median BDI scores improved in all groups over the trial duration, the changes were similar between groups ($\chi 2(2) = 0.0533$, $p = 0.974$). The results suggest that treatment effects were not associated with mood changes (Table 7)

5.7. Modified Ashworth Scale (MAS) and Mini-Mental State Examination MMSE

In the same way, distribution of MAS scores did not differ significantly between groups at inclusion, at 30 or at 90 days. Kruskal-Wallis test analysis showed no statistically significant differences in median MAS scores between groups ($\chi 2(2) = 0.971$, $p = 0.6154$) (Table 7).
A repeated-measures ANOVA for MMSE showed no statistically significant differences in the main effect of time, treatment group, nor in the interaction term time*group ($F<1$ for the three analyses) (Table 7).

Table 7: Changes in secondary Assessments

		Combined (n=9)		Fluoxetine (n=7)		Placebo (n=7)		$\chi 2$	*p* value
BDI	Median (min-max)	-1	(-11 – 9)	-0.5	(-10 – 1)	-2	(-6 – 2)	0.053	0.974
MMSE	Mean (SD)	0.33	(2.18)	0.71	(1.11)	0.17	(0.41)		0.399
MAS	Median (min-max)	0.5	(-2 – 3)	1	(-2.5 – 3)	0.25	(-5 – 1)	0.971	0.615

Data is described in median (range) or mean (SD).BDI= Beck Depression Inventory; MMSE = Mini-Mental State Examination; MAS = Modified Ashworth Scale. Kruskal-Wallis Test was used for MAS; one-way ANOVA was used for MMSE. All data are described as changes from Day 0 to Day 90.No intention to treat analysis- only per protocol.

5.8. Adverse events

During the study period two participant were withdrawn from the clinical trial due to a new onset seizure and as the study staff had to be unblinded. Both adverse events were determined to be unrelated to the study interventions. The process was performed according to the guidelines and procedures for unblinding summarized in Methods (page 48).

Regarding safety outcomes, no severe adverse events were reported. Additionally, there were no statistically significant differences in any adverse events between groups. Adverse event rates for TMS are shown in Table 5. For the calculation of adverse events we considered all TMS sessions, including intervention (rTMS) and assessment (single and paired pulse TMS) sessions of all participants (138 assessments over 21 visits).

All participants completed all sham or active rTMS sessions as well as all single and paired pulse TMS sessions without any serious adverse events. There were no statistically significant differences in all adverse event rates between groups (Table 8).

Table 8: rTMS adverse events

	Combined N=186	**Fluoxetine** N=178	**Placebo** N=114	***P value***
Headache, N (%)	4 (2%)	0 (0%)	9 (8%)	0.221
Neck pain, N (%)	14 (8%)	4 (2%)	4 (4%)	0.505
Skin redness, N (%)	0 (0%)	4 (2%)	1 (1%)	0.523
Sleepiness, N (%)	13 (7%)	12 (7%)	21 (18%)	0.184
Trouble concentrating, N (%)	0 (0%)	1 (1%)	0 (0%)	1.000
Acute mood changes, N (%)	0 (0%)	0 (0%)	1 (1%)	0.296

N = number of TMS-related adverse events; (%) = incidence of event (N/total visits).

Likewise, a few mild adverse events were reported for the Fluoxetine group, and there were no statistically significant differences between groups (Table 9).

Table 9: Fluoxetine's adverse events

	Combined N=179	**Fluoxetine** N=169	**Placebo** N=109	***P-value***
Dry Mouth, N (%)	4 (2.2%)	0 (0%)	3 (2.7%)	0.328
Drowsiness, N (%)	4 (2.2%)	0 (0%)	16 (14.6%)	0.093
Insomnia, N (%)	6 (3.3%)	4 (2.3%)	8 (7.3%)	0.071
Blurred vision, N (%)	0 (0%)	2 (1.2%)	4 (3.7%)	0.273
Headache, N (%)	2 (1.1%)	0 (0%)	3 (2.7%)	0.119
Constipation, N (%)	2 (1.1%)	1 (0.6%)	5 (4.6%)	0.230
Diarrhea, N (%)	0 (0%)	1 (0.6%)	4 (3.7%)	0.134
Decreased appetite, N (%)	1 (0.6%)	1 (0.6%)	6 (5.5%)	0.055
Increased body temperature, N (%)	1 (0.6%)	4 (2.3%)	0 (0%)	0.754
Tremor, N (%)	2 (1.1%)	3 (1.8%)	1 (0.9%)	1.000
Yawning, N (%)	4 (2.2%)	2 (1.2%)	18 (16.5%)	0.572
Weight gain, N (%)	1 (0.6%)	2 (1.2%)	4 (3.7%)	0.403

N = number of fluoxetine-related adverse events; (%) = incidence of event (N/total visits).

5.9. Blinding

We performed a blinding assessment by asking subjects to guess whether they had received active or placebo fluoxetine and active or sham rTMS. We used Cohen's kappa coefficient (κ) to measure the degree of agreement between what each subject believed he or she received (Subject's Guess) and what that subject had actually received (Intervention). At the end of the last intervention day (Visit 22), 22 subjects responded to the blinding questionnaire (Table 10)

.

Table 10: Blinding analysis using Cohen's kappa coefficient

		Intervention			Intervention	
		sham rTMS	real rTMS		placebo Fluoxetine	real Fluoxetine
Subject's Guess	sham rTMS	5	2	placebo Fluoxetine	2	9
	real rTMS	8	7	real Fluoxetine	3	8
	κ	0.147		κ	-0.091	
	Std. Err	0.183		Std. Err	0.179	

The kappa values were 0.14 for rTMS and -0.09 for fluoxetine, implying slight and no agreement respectively between Subject's Guess and Intervention. Although different authors disagree on how to interpret kappa values, we followed the most common accepted classification: values < 0 as no agreement, 0–0.20 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1 as almost perfect agreement (137).

5.10. Open label phase

All subjects randomized to the Placebo and Fluoxetine groups (i.e. all those who received sham rTMS) had an option to participate in the open label phase of the trial. In this phase, each subject received 10 sessions of active rTMS; of the 18 subjects randomized to sham rTMS, 12 participated in the open label phase. Since the study staff could not administer fluoxetine, the medication was not included in the open label phase.

In the open label phase, Wilcoxon signed-rank test found a statistically significant median increase in FMA scores (4.5-point difference) after active rTMS treatment (FMA score 44 points) compared to before active rTMS (FMA score 35 points), $z = -3.024$, $p < 0.0005$. Meanwhile, there were no BDI median score differences before and after rTMS (Figure 11).

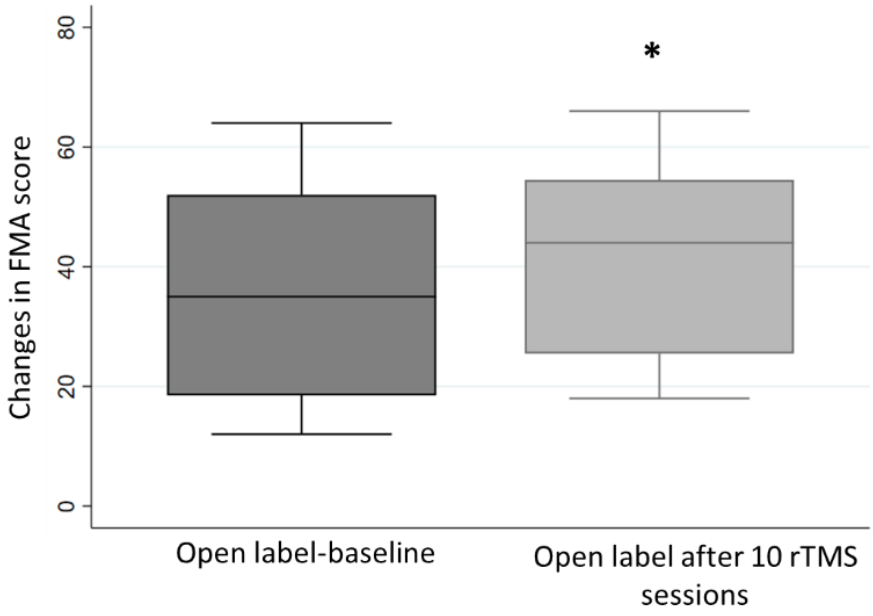


Figure 11: Motor score after open label rTMS

6. DISCUSSION

In the present study on the effects of combined low frequency (inhibitory) contralesional rTMS and fluoxetine therapy in hemiparetic stroke patients, we showed an overall improvement in motor function in the Combined, Fluoxetine and Placebo groups. After adjusting for time since stroke (subacute: <180 days and chronic: >180 days)(138), the Combined group had superior improvements on JTHF as compared to the Fluoxetine and Placebo groups. Conversely, there were no between-group differences on the FMA scale. Meanwhile, the Fluoxetine group had lower motor improvements (on both JTHF and FMA) compared to Placebo, suggesting that fluoxetine might decrease (worsen) motor function improvements in stroke. There were no significant changes in secondary outcomes (BDI, MMSE, MAS) between groups. No major adverse events were reported during or after rTMS and fluoxetine treatments. This was expected as it is in line with previous rTMS studies (139) in stroke patients and as fluoxetine is a well-tolerated drug, especially at the low 20mg/day dose used in this trial.

6.1. Recruitments and enrollment

Our study had an overall randomization yield (proportion of randomized participants among potentially eligible subjects) of 9.3 % (27 of 279 eligible subjects) and a conversion ratio of 10:1 (i.e. 10 subjects screened for each one subject randomized). This is considered average for clinical trials, although our team had to exert tremendous effort on recruitment to reach these numbers. Even after expanding the inclusion criteria, we had to continuously test different strategies and innovate a cost-benefit analysis to optimize our recruitment process. Our cost-benefit analysis showed that online advertising and clinician referrals were the best strategies.

We discuss our recruitment plans and cost-benefit analysis in detail in our upcoming publication (136). We consider this type of analysis to be important for the field, particularly when using innovative approaches such as TMS and tDCS in stroke (140, 141), which have their own set of unique challenges. Additionally, previous stroke trials showed a substantially low recruitment yield and a recent systematic review reported that stroke trial recruitment efficiency has not increased - and might have decreased - over the past 25 years (141). As a result of low recruitment yields, many stroke trials fail to achieve their target sample size, which negatively

affects the validity of study results (142); therefore, it is important to share our recruitment analysis and innovative techniques.

As mentioned above, we were obligated to change our eligibility criteria due to lower recruitment yields than expected and due to changes in medical practice reinforcing this problem (the FLAME (28) trial results led to fluoxetine being commonly prescribed to stroke patients). In August 2015, the inclusion criteria were modified to include not only participants up to 30 days post stroke but also patients up to 2 years post stroke. This change in turn allowed us to include patients already on fluoxetine as we were able to allow for a 5-week washout period. We considered that expanding the time since stroke might not only improve recruitment but also improve our understanding of post-stroke neuroplasticity mechanisms in a broader population, increasing the generalizability of our results. The final analysis presented in this thesis was adjusted for time since stroke in order to address differences in subacute vs. chronic stroke.

The other change was to increase the upper limit of the upper limb FMA score from 45 to 56 points (the new limits were therefore > 11 to 56 points). The upper limb FMA score ranges from 0 to 66 points, but many studies use an upper limit of 56 points or higher as inclusion criteria. However, as some papers consider the minimal clinically important difference (MCID) on upper limb FMA to be either 5 or 10 points, we wanted to allow a change of up to 10 points (143, 144). Additionally, the JTHF score is a continuous scale measuring time in seconds to execute a specific task and can detect fine motor improvements (145). The JTHF score would thus be unaffected by this change in eligibility criteria. However, expanding the FMA inclusion criteria in this way would allow us to increase external validity as well as maintain internal validity or possibly improve it by enhancing recruitment and allowing us to reach our target sample size.

6.2. Combination of fluoxetine and rTMS

We showed that the combination of rTMS and fluoxetine (Combined group) induced larger motor improvements in stroke patients compared to Fluoxetine and Placebo groups; however, contrary to our initial hypothesis, the effects seem to be additive rather than synergistic. One possible explanation is that fluoxetine did not benefit our sample. Supporting this theory, SSRIs did not improve motor function in previous trials on more chronic stroke populations (146, 147). Furthermore, improved mood scores may have mediated fluoxetine's positive effects on motor

function in previous studies; this was not the case in our trial as BDI scores did not differ between groups.

Additionally, in studies evaluating depression scores following combinations of non-invasive brain stimulation and antidepressants (135), a superior and synergistic effect of the combined group was only seen when there was a significant antidepressant effect. Therefore, the superior effect of the Combined group in our study may be explained by rTMS overcoming fluoxetine's unfavorable motor function effects in a subacute to chronic stroke population. This would explain the Combined group's relatively modest motor benefits, especially when compared to recent clinical trials and a meta-analysis on low frequency rTMS in stroke (83, 126, 134, 148-155).

6.3. Differences in motor function outcomes (JTHF vs. FMA)

Motor function improved significantly in the Combined group, however the improvement was only observed in the JTHF scale and not the FMA. This can be explained by differences between the two scales. JTHF evaluates time to perform objective hand function tasks that are usually applied during daily living (156-158); as a continuous scale it is more sensitive to minor changes, and even compensation strategies such as utilizing different body parts might increase performance time. Therefore, the JTHF might be more reflective of overall function compared to the FMA.

The FMA is an ordinal scale that rates movement performance as: not correct, mostly correct or equal to the unaffected limb (0, 1 or 2 points respectively); therefore, it has low sensitivity to measure specific gains in motor function (159). Hand function plays an important role in our trial, however, although JTHF correlated moderately with each of the FMA full score and the FMA wrist and hand subscore, there were no differences between two correlation coefficients (160). The FMA does not measure individual finger movements, which may underestimate distal motor disabilities and any recovery (160) that is enhanced by rTMS. Meanwhile, finger movement and distal coordination are reflected in the tasks performed when measuring JTHF scores. Therefore, the observed JTHF improvement and lack of FMA improvement (despite JTHF's moderate correlation with FMA full and hand/wrist subscores) could be explained by what those scales actually measure, and the low sensitivity of the FMA compared to JTHF.

6.4. Effects of fluoxetine on motor function

In our study, the Fluoxetine group had less motor improvements in both JTHF and FMA scales compared to the Placebo group. Although this contrasts with our original hypothesis, considering more recent studies, it is not particularly surprising given the fact that few RCTs actually tested fluoxetine effects on motor function, and that those RCTs were mainly in acute stroke. Therefore, there could be several reasons for our results.

One possible reason is the difference in time since stroke when comparing our trial to the positive effects seen in the FLAME trial (28). While the FLAME trial included only acute stroke patients and gave them fluoxetine or placebo 5 to 10 days after the stroke, our population ranged from 11 to 725 days since stroke, with a mean of 297 days since stroke overall. Nevertheless, our results suggest that fluoxetine hindered motor function improvements even when adjusted for time since stroke. One caveat is that due to feasibility, time since stroke was analyzed as a categorical measure and potentially underpowered. That said, other studies on SSRIs in acute stroke also showed no significant effects on motor function (161-164); therefore, the effects of fluoxetine in stroke remain unconfirmed. If the effects of fluoxetine are confirmed to be beneficial in acute stroke, the mechanism may be mediated by its anti-inflammatory, angiogenesis-inducing and antioxidant properties that are more relevant for acute than chronic stroke (33, 41-43, 165). If that were the case, then combining fluoxetine with rTMS would not be optimal.

Mood effects might be another reason that fluoxetine led to improved results in other studies but not ours (aside from time since stroke), as our results showed no significant mood changes. Mood and attention can have major negative repercussions on learning and memory, two fundamental processes that influence motor recovery (166). In fact, the FLAME study showed a significant difference in depression scores (Montgomery–Åsberg Depression Rating Scale) between fluoxetine and placebo groups (28), although it is unclear whether mood changes correlated with motor function improvements.

To date, the mechanisms by which fluoxetine may favor motor rehabilitation remains unclear. Our initial hypothesis was that fluoxetine could modulate the excitatory-inhibitory network balance, thus promoting motor recovery in later phases of stroke (12). Although this is an

exploratory study with a small sample size, our results suggest that fluoxetine has more complex mechanistic effects on the excitation-inhibition balance in subacute to chronic stroke.

6.5. Adverse events

In our study there were no between-group differences in the number of TMS and/or fluoxetine adverse events. All reported adverse events were classified as minimal or mild, with no severe or serious adverse events reported.

Overall, rTMS is a safe technique and leads to minor and transient side effects such as local scalp pain, neck pain, transient headache, or toothache. Headaches induced by rTMS can be easily treated with acetaminophen, and typically disappear shortly after stimulation. As long as safety consensus guidelines (co-authored by the principal investigator of this study) are followed, rTMS is very safe (139). Although high frequency rTMS has rarely been associated with a possible risk of seizure (not epilepsy – that is, there is no continued predisposition to seizures once rTMS is discontinued), the 10-year prevalence of seizure was described as "extremely rare" according to the safety and ethical consensus guideline on rTMS (167). The literature has even shown a predominantly protective effect of low frequency rTMS against seizures, while showing a "possible" 1.4% crude risk of seizure with rTMS application in epileptic populations (139). Of note, we excluded patients with epilepsy from our study.

In the case of the one participant that had a seizure during the study, the seizure occurred while driving in the week following the first follow-up visit (i.e. several days after even single and paired pulse TMS), and the subject had been assigned to Placebo (sham rTMS and placebo fluoxetine). The safety committee evaluated the adverse event and determined that it was not related to the study. This patient had a history of left middle cerebral artery stroke nearly 2 years earlier, and epilepsy is one of the possible complications of stroke. The second participant who was dropped out from the study because of an adverse event, he reported to be light-headed and confused during the baseline questionnaires assessments – all safety procedures required were performed and the final diagnosis showed that the participant had a hypoglycemia crisis. The participant had been randomized, however did not received any intervention prior this event.

Finally, the fluoxetine dose used in this clinical trial (20mg) is the standard dose currently used off label in several rehabilitation centers to promote motor recovery after stroke. Its safety profile

has been also established in several studies in psychiatric populations. In our clinical trial, the most common adverse events reported were drowsiness, insomnia and yawning, and those were more common in the Placebo group. By comparison, the most common adverse events in the FLAME study were transient digestive disorders and insomnia, and those were observed in the active group.

6.6. Open label phase

FMA scores improved significantly during the open-label phase. After 10 open label rTMS sessions, all 12 subjects had improved FMA scores (a difference of 4.5 points compared to their open label baseline). However, those same patients had improved by 9.5 points at Day 90 during the blinded phase of the trial, when they had received only fluoxetine or placebo (and sham rTMS). Interestingly, the subjects who had been previously assigned to the Fluoxetine group showed lower FMA improvement in the open label phase when compared to the group previously assigned to Placebo; the difference was of only one point, less than MCID. It is important to note that no washout period was required before starting the open label phase, and that subjects often immediately entered into the open label phase after completing the blinded phase. Therefore, although we could argue that fluoxetine may have influenced the results in a detrimental manner, this cannot be confirmed considering the small non-clinically significant difference and the intrinsic bias present in open label studies (e.g., not all subjects who received sham rTMS decided to come for the open label phase, due to varied reasons such as commute and lack of compensation). As safety and tolerability are already well established for rTMS trials, the open label phase was developed by IRB request to allow all participants to receive active rTMS intervention.

6.7. Placebo effects

In the present study, Placebo outperformed the Fluoxetine group in motor improvements, suggesting that fluoxetine might hinder motor recovery; however, there are other potential factors contributing to the apparent placebo response.

One possible factor is that the placebo response was a result of spontaneous recovery, as participants randomized to Placebo were at earlier stages post stroke. Additionally, the Placebo group showed a superior response in subacute compared to chronic stroke. Natural recovery,

particularly in earlier stages of stroke, and functional fluctuations may play a role in apparent placebo effects as treatment expectations may activate dopaminergic pathways leading to placebo responsiveness (168).

Both lesion size and location may drive the capacity for motor recovery, due to the role of perilesional tissue recruitment. Additionally, stroke location may also influence placebo response, as prefrontal cognitive processing may boost its expression (169). In our study, the Combined and Fluoxetine groups had mostly MCA infarcts, while the Placebo group had more posterior circulation strokes, sparing prefrontal cortical regions, which is potentially consistent with our line of reasoning.

To date, the mechanisms of placebo effects are not well elucidated. Several recent trials have attempted to better understand placebo effects in sham-control rTMS studies (170-172). Irrespective of the type of coil, frequency or location of rTMS the real effects are always associated with psychological and sensory effects that can be difficult to distinguish clearly (hence the need for sham-control trials). A distinct clicking sound is produced by the TMS coil when the pulse is triggered (139, 173), and the magnetic field can stimulate the skin leading to somatosensory effects, aside from possible nerve and muscle stimulation resulting from the common side effect of facial twitching. Therefore, sham rTMS approaches aim to mimic these effects without giving actual stimulation in order to maintain blinding. As shown by the blinding questionnaire, our sham approach was particularly effective, which may have unintentionally enhanced the placebo response (in both rTMS sham groups) by activating the dopaminergic systems of learning (174). However, as the Placebo and Fluoxetine groups both received sham rTMS, and as the Combined group had better motor outcomes than both, the placebo response is insufficient to explain our results.

Finally, the placebo effects of fluoxetine and other antidepressants are well described; yet, their mechanisms remain unclear. Even though most of these analyses were performed in depression studies, drug vs. placebo effect size differences are small enough to be clinically insignificant, suggesting that placebos might work as well as the real drugs (174). This is concerning as – unlike rTMS - antidepressants have significant side effects and are widely prescribed. In our study, the fluoxetine treatment did not seem to be beneficial (and may have been tentatively

detrimental at this stage); however, there is no evidence to refute or accept the hypothesis that the placebo pill led to a significant placebo effect.

Therefore, in future trials using rTMS and SSRIs, different methodologies should be used to better control for these effects. For example, the use of a control group (no intervention) could shed some light on the effects of placebo rTMS and placebo fluoxetine in motor rehabilitation after stroke.

6.8. Comparing the motor improvements of our study with the literature

Despite several attempts in trying to model and predict motor function recovery after stroke (i.e. proportional recovery model) as well as research to find the "best treatment" approach, motor rehabilitation after stroke frequently leads to heterogenous results. To date, the mechanisms behind motor impairment and recovery after stroke are not fully understood and no "optimum/ideal treatment protocol" is described.

Most of the literature points towards the role of interdisciplinary rehabilitation strategies, including non-invasive brain stimulation techniques such as rTMS (as in this trial). However, recent meta-analyses and reviews discussing the role of rTMS in motor function rehabilitation after stroke expressed concern as effects sizes are small and as recent trials reported no differences between rTMS and control groups. Even though our trial's primary hypothesis of synergistic effects between rTMS and fluoxetine was not confirmed, we were able to show small but significant effects of the combined intervention compared to placebo. As we used adjusted means due to the confounding effect of time since stroke, it was statistically impossible to calculate an effect size for our study. To better compare our trial to recent literature, I performed a small systematic review of published stroke rTMS trials. In this review I included only RCTs that also used a sham rTMS group as well as JTHF (Table 11) or FMA (Table 12) upper limb scales as motor outcomes.

The present analysis provides a state of the art summary on the use of various rTMS protocols to improve upper limb motor recovery post-stroke. A better understanding of different protocols and their effects on motor outcomes can help us optimize the therapeutic effects of this intervention.

6.8.1. Combined InterventionsIn total 17 studies (6 using JTHF and 11 using FMA outcomes) were included in this review on different rTMS protocols. In the next subsections I will briefly summarize the results of this review, comparing them to the protocol used in our clinical trial

Recent studies have increasingly used rTMS as an add-on treatment (rather than as monotherapy), combining this technique with other pharmacological or behavioral therapies aiming to increase their effects. Some authors argue that rTMS alone cannot produce enough physiological changes to manifest in behavioral improvements. They argue that rTMS can cause temporary brain state changes facilitating skill acquisition and optimizing learning induced by standard therapies, but that it cannot act alone. The vast majority of the trials summarized in Tables 11 and 12 combined rTMS with another intervention, mostly with some form of motor training (e.g., physical or occupational therapy). However, motor improvement was not observed in most cases. That said, most studies using JTHF showed significant motor improvement while most studies using FMA did not.

The absence of rTMS response can be related to several factors, including inter- and intra-subject variability, differences in multiple aspects of stimulation protocols (e.g., location, frequency, duration, interval). Additionally, researchers have recently begun questioning the effectiveness of behavioral therapies, which can be difficult to standardize for RCTs and can thus be challenging to validate. If the main principle is that rTMS can enhance the effects of a behavioral/pharmacological therapy, but that therapy has little impact on motor outcomes as monotherapy, it is possible that augmentation by rTMS or another therapy may not lead to noticeable effects. Conversely, if that therapy is highly effective, it may lead to ceiling effects that cannot be further augmented by rTMS. The ideal scenario would be to find a synergistic combination therapy to optimize motor outcomes post-stroke.

As previously discussed, synergistic effects of a combined intervention may only become apparent when the pharmaco/behavioral therapy is able to show clinically meaningful differences acting on its own (depending on the degree of synergy). In our study, the Fluoxetine group led to less motor improvement than Placebo, and despite the biases of open label trials, the formerly Fluoxetine group did slightly worse than the formerly Placebo group even when receiving active

rTMS (at a time when fluoxetine effects could have been ongoing due to the lack of a washout period).

Combined therapy effects aside, it is important to fully understand which therapy primes the other and drives motor outcome changes, as well as the parameters required to have a clinical effect. Avenanti et (175) al found that low frequency rTMS applied before physical therapy (PT) lead to greater and more enduring motor function improvements in chronic stroke patients compared to rTMS applied after PT. Nevertheless, the recently published NICHE study showed no differences between the groups when combining low frequency rTMS before motor training in stroke patients (that said, as both active and sham rTMS groups improved and considering the heavy load of PT, the groups may have reached a ceiling effect due to PT, even if that effect was modest). Hence, the need for more information regarding the relationship between rTMS combined with additional therapies and the brain pathways is evident.

6.8.2. rTMS parameters and experimental design

It is important to point out that rTMS parameters vary a great deal between studies, and it is unclear which parameters can lead to an optimal dosage and under which circumstances. To date, most trials used low frequency rTMS over the unaffected M1 (as in our study). Although both low frequency rTMS of the unaffected hemisphere and high frequency rTMS of the affected hemisphere could in theory enhance motor function, some trials comparing both techniques showed superior effects of low frequency M1 rTMS.

Most studies focused on M1 rTMS to treat motor dysfunction, but some investigated rTMS of other areas such as the premotor cortex. Based on previous literature, our study targeted the healthy M1 aiming for a large effect on motor rehabilitation; however, our motor function effects were modest, though no more so than in other rTMS studies. Our hypothesis that fluoxetine would lead to synergistic effects with rTMS was not confirmed, but considering the heterogeneity of rTMS studies in general, the search continues for optimal combination therapies and rTMS protocols for specific clinical outcomes.

This systematic review showed high variability in number of sessions, intervals, and pulse frequency among other parameters, but the exact effects of these variables remains to be seen.

6.8.3. rTMS to improve motor function after stroke

In the review, 52% of the trials did not report any significant motor improvements between the active and sham rTMS groups. However, as in our trial, most studies using JTHF reported improved motor function (4 out of 6 studies) while most studies using FMA did not (10 out of 17 papers showed no differences between groups). As previously discussed, careful selection of motor outcomes as well as timepoints relative to the research question may help optimize future clinical trials. Additionally, it will be important to better understand the effects of different rTMS protocols on strokes in different locations, of different sizes and occurring at various points in time, along with other confounders. These factors are typically not addressed in clinical trials included in this review. In our trial, the Placebo group had less time since stroke compared to the other groups; this finding was not statistically significant but did affect the model showing that it was a confounder and necessitating that we adjust our results for this factor. Another potential strategy is to have a stratified randomization but considering that rTMS studies tend to have small sample sizes, this may not be feasible.

A main advantage of rTMS and reason enough to continue investigating its effects is that rTMS has great potential for individualization. Once its effects are better understood in the context of the underlying pathophysiology, it can be used to tailor therapy to specific patient needs and targeted to responders.

In summary, there is much to be investigated in order to optimize rTMS as a therapy, including various stimulation parameters, study designs and patient selections. However, rTMS holds promise as a safe therapeutic that may be tailored to individual patient needs.

Table 11: JTHF in rTMS stroke RCTs review

Author	Publication Year	Design	Stroke type	Time since stroke	TMS parameters	additional therapy	Study groups	N	Comments
Pinto et al. submitted	submitted	Parallel	I	41 weeks	1 Hz, 1200 pulses (20 min), 100% rMT, M1 unaffected, 18 sessions	Fluoxetine	sham rTMS+placebo fluoxetine	8	There were differences in motor improvement between the sham rTMS+placebo fluoxetine and the active rTMS+fluoxetine
							sham rTMS+fluoxetine	10	
							LF-rTMS+fluoxetine	9	
Conforto et al.(176)	2012	Parallel	I	4 weeks	1 Hz, 1500 pulses (25 min), 90% of rMT, M1 unaffected, 10 sessions	Occupational therapy	sham rTMS+OT	14	Motor improvement only observed in the active rTMS group, however no comparison with the sham
							LF-rTMS+ OT	15	
Lüdemann-Podubecká et al.(177)	2016	Crossover	Both	4 weeks	1 Hz, 900 pulses (15 min), 100% rMT, PMd unaffected, 2 sessions	NA	sham rTMS	10	There were differences in motor improvement between the sham and active rTMS group
							LF-rTMS	10	

Author	Publication Year	Design	Stroke type	Time since stroke	TMS parameters	additional therapy	Study groups	N	Comments
Chang et al.(178)	2012	Parallel	Both	40 weeks	10 Hz, 100 pulses (20 min), 80% rMT, M1 affected, 10 sessions	Motor finger training	sham rTMS+MT	8	Differences between active and sham stimulation were observed only the simulated feeding task of the JTHF test
							HF-rTMS + MT	9	
Avenanti et al.(179)	2012	Parallel	Both	126 weeks	1 Hz, 1500 pulses (25 min), 90% rMT, M1 unaffected, 10 sessions	Physiotherapy	sham rTMS	14	Active groups showed better improvements than sham rTMS; LF-rTMS before PT improve more than rTMS after PT
							LF-rTMS before PT	8	
Fregni et al.(180)	2006	Parallel	I	176 weeks	1 Hz, 1200 pulses (20 min), 100% rMT, M1 unaffected, 5 sessions	NA	sham rTMS	5	There were differences in motor improvement between the sham and active rTMS group
							LF-rTMS	10	

LF: Low Frequency; HF: High Frequency; rTMS: repetitive Transcranial Magnetic Stimulation; M1: primary motor cortex; rMT: Resting motor threshold; aMT: active motor threshold; Hz: Hertz; I: Ischemic stroke; Both: Ischemic and Hemorrhagic; iTBS: intermittent theta burst stimulation

Table 12: FMA stroke RCT reviews

Author	Publication Year	Design	Stroke type	Time since stroke	TMS parameters	additional therapy	Study groups	N	Comments
Pinto et al. Submitted	submitted	parallel	I	41 weeks	1 Hz, 1200 pulses (20 min), 100% rMT, M1 unaffected, 18 sessions	fluoxetine	sham rTMS+placebo fluoxetine	8	There were no differences between the groups
							sham rTMS+fluoxetine	10	
							LF-rTMS+fluoxetine	9	
Sung et al.(181)	2013	parallel	I	1 week	1 Hz, 600 pulses, 90% rMT, M1 unaffected and/or iTBS, 600 pulses, 80% aMT, M1 affected, 20 sessions	passive limb movement +conventional therapy and medical treatment	sham rTMS +sham iTBS	14	There were differences in motor improvement between the sham and active rTMS groups
							LF-rTMS+iTBS	15	
							LF-rTMS+sham iTBS	12	
							shan-rTMS+iTBS	13	

Author	Publication Year	Design	Stroke type	Time since stroke	TMS parameters	additional therapy	Study groups	N	Comments
Matsuura et al.(182)	2015	parallel	I	1 week	1 Hz, 1200 pulses (20 min), 100% rMT, M1 unaffected, 5 sessions	NA	sham rTMS	10	There were differences in motor improvement between the sham and active rTMS group
							LF-rTM	10	
Du et al.(183)	2016	Parallel	I	1 week	3 Hz, 1200 pulses, 80-90% rMT, M1 affected or 1 Hz, 1200 pulses (20 min), 110% rMT, M1 unaffected, 5 sessions	Physical therapy	Sham rTMS	23	There was significant motor improvement between the sham and LF rTMS group
							HF-rTMS + PT	23	
							LF-rTMS+ PT	23	
Chang et al.(184)	2010	parallel	I	2 weeks	10 Hz, 1000 pulses,90% rMT, M1 affected, 10 sessions	Motor practice	sham rTMS	10	There were no differences between the groups
							HF-rTMS+MP	18	
Hosomi et al.(185)	2016	parallel	Both	5 weeks	5Hz,500 pulses, 90% rMT M1 affected, 10 sessions	conventional, physical and occupational therapy	sham rTMS	21	There were no differences between the groups
							HF-rTMS	18	

Author	Publication Year	Design	Stroke type	Time since stroke	TMS parameters	additional therapy	Study groups	N	Comments
Li et al.(186)	2016	Parallel	I	6 weeks	1 Hz, 1000 pulses (20 min), M1 unaffected or 10 Hz, 1350 pulses (20 min) M1 affected, 80% rMT, 10 sessions	Occupational therapy	sham rTMS + OT	42	There were differences in motor improvement between the sham and active rTMS groups and no differences between LF and HF rTMS
							LF-rTMS + OT	42	
							HF-rTMS + OT	43	
Wang et al.(187)	2014	Parallel	I	18 weeks	1 Hz + 10 iTBS or 10 iTBS stim + 1 Hz, 600 pulses (10 min), rTMS-90% rMT and iTBS 80%aMT, rTMS on M1 unaffected and iTBS M1 affected;	Conventional physiotherapy	Sham rTMS + sham iTBS	16	There were differences in motor improvement between the sham and active rTMS group
							LF-rTMS + iTBS stim (Group A)	17	

Author	Publication Year	Design	Stroke type	Time since stroke	TMS parameters	additional therapy	Study groups	N	Comments
Wang et al.(188)	2014	Parallel	Both	29 weeks	1 Hz, 600 pulses, 90% of rMT, M1 or PMd unaffected, 10 sessions	NA	sham rTMS	14	There were differences in motor improvement between the sham and active rTMS group
							LF-rTMS M1	16	
Ji et al(189).	2014	Parallel	Both	31 weeks	10 Hz, 15 min, M1 affected, 18 sessions	mental practice and Physiotherapy	Sham-rTMS + mental practice + physiotherapy	16	There were no differences between the groups
							HF-rTMS + mental practice + physiotherapy	16	
Harvey et al.(190)	2018	parallel	Both	12-48 weeks	1 Hz, 900 pulses (15 min), 100%rMT, 18 sessions	Task-oriented rehabilitation therapy	sham rTMS+OR	67	There were no differences between the groups
							LF-rTMS+OR	132	
Özkeskin et al.(191)	2016	parallel	I	70 weeks	1 Hz, 1500 (25 min), M1 unaffected, 10 sessions	Brunnstrom hand manipulation (BHM) and upper extremity exercises.	sham rTMS	11	There were no differences between the groups
							LF-rTMS	10	

Author	Publication Year	Design	Stroke type	Time since stroke	TMS parameters	additional therapy	Study groups	N	Comments
Vaziri et al.(192)	2014	parallel	*	94 weeks	1 Hz, 20 min, 80% rMT,M1 affected, 10 sessions	Rehabilitation program	sham rTMS+ rehab program	6	There were no differences between the groups
							LF-TMS+ rehab program	6	
Etoh et al.(193)	2013	cross-over	Both	120 weeks	1 Hz, 240 pulses (4 min), 90% rMT, M1 unaffected, 10 sessions	repetitive facilitation +voluntary training	sham rTMS	9	There were no differences between the groups
							LF-rTMS	9	
Barros et al(194).	2014	Parallel	Both	213 weeks	1 Hz, 1500 pulses, 90% rMT, M1 unaffected, 10 sessions	Physiotherapy	sham rTMS + PT	10	There were no differences between the groups
							LF-rTMS+ PT	10	
Rose et al.(195)	2014	parallel	NR	240 weeks	1 Hz, 1200 pulses (20 min), 100% rMT, M1 unaffected, 16 sessions	functional task practice	sham rTMS+ FP	10	There were no differences between the groups
							LF-rTMS+FP	9	

Author	Publication Year	Design	Stroke type	Time since stroke	TMS parameters	additional therapy	Study groups	N	Comments
Meng et al.(196)	2017	parallel	I	NR	1 Hz, 1800 (30 min), 90% rMT, M1unaffected, 14 sessions	NA	LF-rTMS	10	There were differences in motor improvement between the sham and active rTMS group
							sham rTMS	10	

LF: Low Frequency; HF: High Frequency; rTMS: repetitive Transcranial Magnetic Stimulation; rMT: Resting motor threshold; aMT: active motor threshold; M1: primary motor cortex; Hz: Hertz; I: Ischemic stroke; Both: Ischemic and Hemorrhagic; iTBS: intermittent theta burst stimulation

7. LIMITATIONS

This mechanistic study has some limitations, such as the small and heterogeneous sample that reduces the likelihood of detecting treatment effects, as well as the nonsignificant but confounding differences in time since stroke between Placebo and other groups. Future clinical trials accounting for stroke chronicity can better evaluate fluoxetine's effects on stroke motor rehabilitation. As this was not a full factorial trial and no comparisons were made with an rTMS-only group, we cannot further investigate possible unfavorable effects of fluoxetine. Additionally, a long-term follow up after the end of the treatment would be required to assess the duration of effects. Another potential limitation of our trial is that the sham coil has been known to induce low strength electric fields that can reportedly reach up to 7.2% of the intensity of the active coil (197). Aside from the coil, the electrode used in this particular rTMS placebo method induces an electrical current which, despite being very small, could potentially lead to cortical activation; we must take this into consideration when analyzing the motor recovery improvements seen in the rTMS placebo groups (although at least the sham is reliable).

8. CONCLUSION

Combined fluoxetine and low-frequency rTMS treatment of the unaffected hemisphere improved motor function in stroke beyond the effects of fluoxetine alone and placebo. Fluoxetine may have had a relatively detrimental effect, leading to decreased motor function improvements compared to placebo; it is possible that rTMS overcame this effect in the combined group. However, further mechanistic stroke trials are needed to clarify the effects of both treatments and to investigate optimal parameters for rTMS in specific patient populations.

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