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Comprehensive Upper Limb Rehabilitation: Examining the Synergistic Effects of rTMS and MI-Based NFB Therapy in Stroke Patients

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A mi familia, amigos y a vosotros, queridos pacientes

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Publications and participation in national and international conferences:

Publications:

- Sánchez-Cuesta FJ, González-Zamorano Y, Arroyo-Ferrer A, Moreno-Verdú M, Romero-Muñoz JP. Repetitive transcranial magnetic stimulation of primary motor cortex for stroke upper limb motor sequelae rehabilitation: A systematic review. NeuroRehabilitation. 2023;52(3):329-48. https://doi.org/10.3233/NRE-220306

- Sánchez-Cuesta, F.J.; Arroyo-Ferrer, A.; González-Zamorano, Y.; Vourvopoulos, A.; Badia, S.B.i.; Figuereido, P.; Serrano, J.I.; Romero, J.P. Clinical Effects of Immersive Multimodal BCI-VR Training after Bilateral Neuromodulation with rTMS on Upper Limb Motor Recovery after Stroke. A Study Protocol for a Randomized Controlled Trial. Medicina 2021, 57, 736. https://doi.org/10.3390/medicina57080736

- Sánchez Cuesta FJ, González-Zamorano Y, Moreno-Verdú M, Vourvopoulos A, Serrano IJ, Del Castillo-Sobrino MD, Figueiredo P, Romero JP. Effects of motor imagery-based neurofeedback training after bilateral repetitive transcranial magnetic stimulation on post-stroke upper limb motor function: an exploratory crossover clinical trial. J Rehabil Med. 2024 Mar 7;56: jrm 18253. https://doi.org/10.2340/jrm.v56.18253

Conferences and seminars:

XI World Congress for Neurorehabilitation, online, 7-10 october 2020

- Poster: Use of repetitive transcranial magnetic stimulation as an adjunctive therapy in the rehabilitation of the upper limb in cortical ischemic stroke. A research proposal for a double blind and crossed controlled study.

LXXII Reunión anual Sociedad Española de Neurología, Sevilla 17-21 november 2020

- Poster: Uso combinado de estimulación magnética transcraneal repetitiva e imaginería motora como terapias complementarias en la rehabilitación de la extremidad superior en el accidente cerebrovascular isquémico cortical. Protocolo de estudio controlado doble ciego.

4th International Brain Stimulation Conference, Charleston 7-10 december 2021

- Poster: Clinical effects of Immersive Multimodal BCI-VR Training after Bilateral Stimulation with rTMS on Upper Limb Motor Recovery after stroke.

XII World Congress for Neurorehabilitation, Viena, 13-17 december 2022

- Poster: Clinical effects of conventional rehabilitation after bilateral stimulation with rTMS on upper limb motor recovery after stroke.

5th International Brain Stimulation Conference, Lisbon 18-22 february 2023

- Poster: Clinical effects of Immersive Multimodal BCI-VR Training after Bilateral Stimulation with rTMS on Upper Limb Motor Recovery after stroke.

9th Congress of the European Academy of Neurology, Budapest, 1-4 july, 2023

- Poster: Effects of motor imagery with neurofeedback after bilateral stimulation with rTMS on upper limb recovery after stroke.

XVII Congreso Nacional de Fisioterapia AEF, Cáceres, 24-25 november 2023

- Oral Presentation: Efectos clínicos de la imaginería motora-neurofeedback tras EMTr sobre la función motora del miembro superior post-ictus.

RESUMEN

Introducción: La Organización Mundial de la Salud (OMS) definió el accidente cerebrovascular (ACV) como una alteración cerebral vascular que produce síntomas neurológicos de inicio brusco, con duración de más de 24 horas. Representa una de las principales causas de discapacidad y muerte en España y a nivel mundial.

Después de un semestre del evento cerebro vascular, más del 60% de los participantes no pueden realizar tareas esenciales de la vida diaria debido a problemas motores entre los cuales son muy significativos aquellos que afectan la mano.

Existen varias técnicas que se usan para complementar la rehabilitación convencional incluyendo en la excitabilidad cerebral para potenciar sus efectos. Entre estos están las técnicas de neuromodulación cerebral no invasiva exógenas y endógenas. La Estimulación Magnética Transcraneal Repetitiva (EMTr) es una de las técnicas de neuromodulación exógena que se han probado exitosamente en la rehabilitación motora del ictus, y por otro lado el neurofeedback y la imaginería motora son dos técnicas de neuromodulación endógena que también han mostrado resultados prometedores, mejorando aspectos como la fuerza, la destreza y la funcionalidad del miembro superior afectado. Hasta la fecha existen pocos estudios que prueben el efecto de la combinación de estrategias endógenas y exógenas en la rehabilitación del ictus.

Objetivo: Se investigan los efectos de un protocol de neuromodulación no invasive multimodal para potenciar la rehabilitación de las secuelas motoras en miembro superior de pacientes con un ACV en fase crónica

Métodos y Resultados: En primer lugar, se realizó una revisión sistemática de la efectividad de la EMTr en la recuperación del miembro superior tras un ACV. Aunque los resultados enfatizan falta de de ensayos clínicos homogéneos que permitan un meta análisis extrapolable, se evidencia un efecto positivo de la EMTr en los síntomas motores de miembro superior secundarios a un ictus.

En segundo lugar, se diseñó un ensayo clínico con el objetivo de potenciar los efectos motores y funcionales de la EMTr aplicada bilateralmente, usando el principio de rivalidad interhemisférica. Se combina este protocolo con neuromodulación endógena con un entrenamiento multimodal de IM basado en NFB usando realidad virtual.

En tercer lugar, se lleva a cabo el ensayo clínico en 20 pacientes con ACV subagudo o crónico con algunas modificaciones respecto al protocolo inicialmente diseñado para aumentar su calidad. Sus resultados revelaron que la IM basada en NFB puede mejorar los efectos de la EMTr bilateral en la función y sensibilidad de la extremidad superior

con persistencia de los efectos de al menos un mes después de la finalización del protocolo. Se evidenció además, un impacto diferencial en la mejora de la función en comparación con la fuerza con el uso de la IM, resaltando la importancia de planes terapéuticos personalizados.

Finalmente se hizo un análisis avanzado de los cambios neurofisiológicos producidos por el protocolo usando los registros EEG y otros datos de excitabilidad cortical registrados como variables secundarias. Se identificaron cambios importantes en la inhibición intracortical en el hemisferio no afectado como producto de la estimulación con rTMS, además se observaron patrones distintivos en la asimetría cerebral en reposo que no fue evidente durante las tareas motoras.

Se observa un impacto significativo de la rTMS en la modulación de la desincronización relacionada con eventos (ERD), durante la ejecución de las tareas de NFB e IM del protocolo de neuromodulación aplicado.

No se observan cambios evidentes en la conectividad cerebral ni medida como simetría interhemisférica ni como diferencias de entropía que puedan usarse para correlacionar con efectos clínicos

En conclusión, la combinación de rTMS con IM basado en NFB muestra potencial en mejorar la función motora y la sensación somatosensorial del miembro superior afecto en pacientes con accidente cerebrovascular subagudo y crónico. Esto sugiere la posibilidad estrategias de rehabilitación personalizadas mejoradas. Sin embargo, es esencial perfeccionar los protocolos y abordar las limitaciones del estudio a través de una investigación adicional.

ABSTRACT

Introduction: The World Health Organization (WHO) defines stroke as a cerebrovascular alteration resulting in sudden onset neurological symptoms lasting over 24 hours. It stands as a major cause of disability and death both in Spain and globally. Following a six-month period post-stroke, over 60% of participants experience difficulties performing essential daily tasks due to motor impairments, particularly those affecting the hand.

Various techniques complement conventional rehabilitation, focusing on enhancing cerebral excitability to potentiate its effects. Non-invasive exogenous and endogenous cerebral neuromodulation techniques are among these strategies. Repetitive Transcranial Magnetic Stimulation (rTMS) is an exogenous neuromodulation technique successfully tested in stroke motor rehabilitation. Additionally, neurofeedback and motor imagery represent endogenous neuromodulation techniques, also displaying promising outcomes in improving aspects such as strength, dexterity, and functionality of the affected upper limb. However, few studies have investigated the combined effects of endogenous and exogenous strategies in stroke rehabilitation.

Objective: This research explores the effects of a multimodal non-invasive neuromodulation protocol to enhance motor recovery in the upper limb of chronic stroke patients.

Methods and Results: Firstly, a systematic review assessed the effectiveness of rTMS in upper limb recovery post-stroke. Although the results emphasize the lack of homogeneous clinical trials for a conclusive meta-analysis, a positive effect of rTMS on stroke-related upper limb motor symptoms was evident.

Secondly, a clinical trial was designed to enhance motor and functional effects of bilateral rTMS through the interhemispheric rivalry principle. This protocol combined endogenous neuromodulation with multimodal training of Motor Imagery (MI) based on Neurofeedback (NFB) using virtual reality.

Thirdly, the clinical trial was conducted with 20 subacute or chronic stroke patients, introducing some modifications to the initially designed protocol for improved quality. Findings revealed that NFB-based MI could enhance the effects of bilateral rTMS on upper limb function and sensitivity, persisting for at least one-month post-protocol completion. Differential impacts were noted in function improvement compared to strength with MI, highlighting the importance of personalized therapeutic plans.

Finally, an advanced analysis of neurophysiological changes induced by the protocol using EEG recordings and other cortical excitability data as secondary variables was conducted. Significant alterations in intracortical inhibition in the unaffected hemisphere due to rTMS stimulation were identified. Distinct patterns in resting cerebral asymmetry were observed, not evident during motor tasks.

A significant impact of rTMS on modulating Event-Related Desynchronization (ERD) during the execution of NFB and MI tasks in the applied neuromodulation protocol was noted. However, no evident changes were observed in brain connectivity, measured as interhemispheric symmetry or entropy differences that could be correlated with clinical effects.

In conclusion, the combination of rTMS with NFB-based MI shows potential in improving motor function and somatosensory sensation in the affected upper limb of subacute and chronic stroke patients. This suggests enhanced possibilities for personalized rehabilitation strategies. Nonetheless, refining protocols and addressing study limitations through further research remains essential.

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ABBREVIATIONS

9HPT	Nine Hole Peg Test
ADLs	Activities Daily Life
AO	Action Observation
ARAT	Action Research Arm Test
BBT	Box and Block Test
BCI	Brain Computer Interfaz
BI	Bartel Index
CMCT	Central Motor Conduction Time
CMTT	Conduction Motor Total Time
CRT	Cognitive Rehabilitation Therapy
cRT	Choice Reaction Time
CSF	Cerebrospinal Fluid
CSP	Cortical Silent Period
CT	Computed Tomography
cTBS	Continuous Theta Burst Stimulation
CVA	Cerebrovascular Accident
ECG	Electrocardiogram
EEG	Electroencefalography
FAS	Functional Ambulation Scale
FIM	Functional Independence Measurement
FMA-TT	Fugl Meyer Assessment-Total
FMA-UL	Fugl Meyer Assessment-Upper Limb
fMRI	Functional Magnetic Resonance Imaging
fNIRS	Functional Near Infrared Spectroscopy
FTT	Finger Tapping Test
GABA	Gamma-aminobutyric acid
HEG	Hemoencefalography
HF-rTMS	High Frecuency-Repetitive Transcranial Magnetic Stimulation
ICF	Intracortical Facilitation
ICI	Intracortical Inhibition
IHI	Interhemispheric Inhibition
ISP	Ipsilateral Silent Period
iTBS	Intermitent Theta Burst Stimulation
JHFT	Jebsen-Taylon Hand Function Test

LACI	Lacunar Infarctions
LENS	Low-Energy Neurofeedback System
LF-rTMS	Low Frecuency-Repetitive Transcranial Magnetic Stimulation
LLDFI	Late-Life Function and Disability Index
LMM	Lineal Mixed Model
LORETA	Low-Resolution Electromagnetic Tomography
LSL	Lab Streaming Layer
LTD	Long Term Depression
LTP	Long Term Potentiation
M	Measurement
M1	Primary Motor Cortex
MAL	Motor Activity Log
MAS	Modified Asworth Scale
MBI	Modified Barthel Index
MEG	Magnetoencephalography
MEP	Motor-Evoked Potencial
MI	Motor Imagery
MI	Motricity Index
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MPC	Medial Premotor Cortex
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
NFB	Neurofeedback
NIHSS	National Institutes of Health Stroke Scale
NMDA	N-methyl-D-Aspartate
NMES	Neuromuscular Electrical Stimulation
NS	Number of Session
NSA	Nottingam Sensory Assessment
NSA-KS	Nottingam Sensory Assessment-Kinesthetic Sensation
NSA-S	Nottingam Sensory Assessment-Stereognosis
NSA-TS	Nottingam Sensory Assessment-Tactile Sensation
PACI	Partial Anterior Circulation Infarctions
PMCT	Peripheral Motor Conduction Time
POCI	Posterior Circulation Infarctions
PPT	Purdue Pegboard Test

PT	Physical Therapy
QEEG	Quantitative Electroencefalography
Ref	Reference
RMT	Resting Motor Threshold
ROM	Range of Motion
rTMS	Repetitive Transcranial Magnetic Stimulation
S1	Somatosensory Cortex
SCP-NF	Slow Cortical Potential Neurofeedback
SD	Estándar Desviation
SICI	Short Intracortical Inhibition
SIS	Stroke Impact Scale
sRT	Simple Reaction Time
SSQOL	Stroke Specific Quality of Life Scale
STEF	Simple Test for Evaluating Hand Function
TACI	Total Anterior Circulation Infarctions
TCI	Transcrallosal Inhibition
TEMPA	Test Evaluant la Performance des Membres supérieurs des Personnes âgées (from French is assessing the upper limb performance of elderly individuals)
TMS	Transcranial Magnetic Stimulation
UL	Upper Limb
V50	The stimulus intensity at which MEP amplitude is 50% of the MEPMAX
VE	Virtual Enviroment
VR	Virtual Reality
WHO	World Health Organization
WMFT	Wolf Motor Function Test

1. INTRODUCTION

1.1. CEREBROVASCULAR ACCIDENT

1.1.1. CONCEPT AND EPIDEMIOLOGY

In the 1970s, the World Health Organization (WHO) defined a cerebrovascular accident (CVA) or stroke as "clinical signs of focal or global alteration of brain function, rapidly developing with a duration of more than 24 hours, and of vascular origin" (1).

There is some controversy regarding the concept, as international associations such as the American Stroke Association and the World Stroke Organization call for an update of the definition in accordance with new findings and advances in the field of stroke.

The Stroke Council of the American Heart Association, along with the American Stroke Association, convened a panel of experts to develop a consensus document with the aim of providing an updated definition of stroke for the 21st century. In this report, central nervous system infarction is defined as the death of brain, spinal cord, or retinal cells attributable to ischemia, based on neuropathological, neuroimaging, and/or clinical evidence of permanent injury. Central nervous system infarction encompasses ischemic stroke with evident symptoms, silent infarction without known symptoms, intracerebral hemorrhage, and subarachnoid hemorrhage (2).

In a systematic review that investigated the global, regional, and national burden of neurological disorders from 1990 to 2016, it was concluded that stroke was the largest contributor to years of life adjusted for disability among all neurological disorders, accounting for 42.2% of the total burden. Similarly, it confirms that in 2016, cerebrovascular disease was responsible for approximately 5.5 million deaths and the loss of 116.4 million years of quality of life worldwide (3).

The Spanish Society of Neurology issued in 2022 a statement on World Stroke Day, stating that stroke is the second leading cause of death in Spain, being the first in women, and the leading cause of acquired disability in adults. Each year, 110,000-120,000 people suffer a stroke in Spain, representing an average incidence ranging from 120 to 350 cases per 100,000 inhabitants per year (4).

In Spain, the adjusted mortality rate for cerebrovascular diseases per 100,000 inhabitants is used as an indicator to assess acute-phase care for stroke patients. Data has been collected over an extensive period (2008 to 2018) to examine the evolution of this indicator since the publication of the Stroke Strategy of the National Health System.

The data reveals a decline in standardized mortality rates for stroke over the course of the study period, with a minimum around 2015 at 20.59 per 100,000 inhabitants. Significant regional variations are evident; however, it is noteworthy that these differences have been diminishing over the series. For instance, in 2008, the range of the rate ranged from a minimum of 20.28 to a maximum of 52.40, whereas in 2018, it fluctuated between 14.99 and 31.61 (Figure 1).

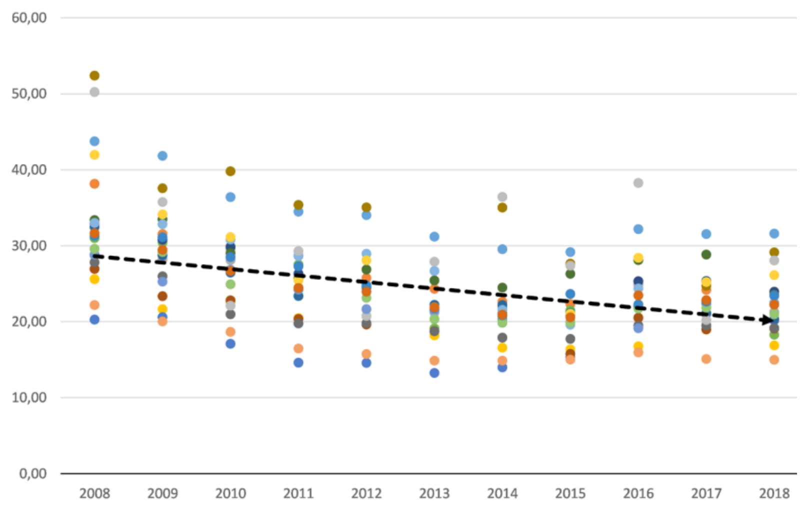

Figure 1. Mortality. Adjusted Rates of Mortality from Cerebrovascular Diseases per 100,000 inhabitants. Trend line and variability (5).

The adjusted mortality rate for cerebrovascular diseases exhibits consistent differences between men and women throughout the study period (Figure 2), with higher mortality rates consistently observed in men (25.58 in men compared to 19.30 in women per 100,000 inhabitants in 2018).

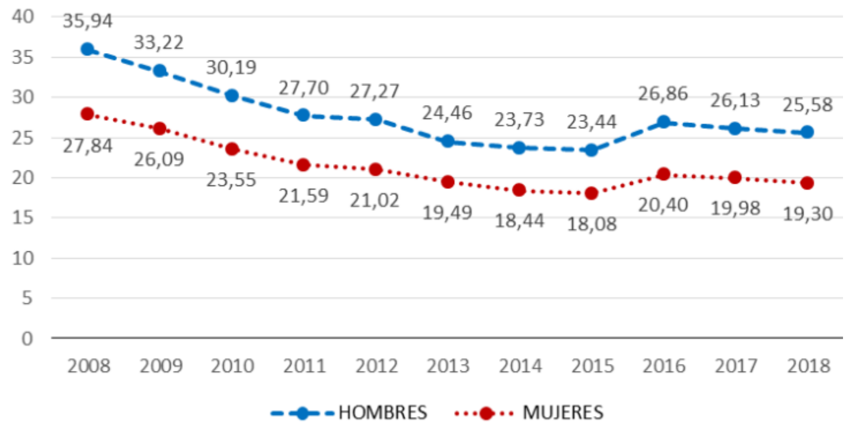

Figure 2. Adjusted Mortality Rates from cerebrovascular diseases per 100,000 inhabitants, by gender. Years 2008-2018(5).

According to the Disability, Personal Autonomy, and Dependency Situations Survey by the National Institute of Statistics, updated in April 2022, there are approximately 361,500 people in Spain living with a disability because of having experienced a stroke.

1.1.2. SOCIOECONOMIC IMPACT

The socioeconomic impact of stroke in Spain is substantial and encompasses various aspects, including healthcare costs, productivity loss, long-term disability, and the burden on families and society at large. Here are some key points regarding the socioeconomic impact of stroke (6,7):

- Healthcare Costs: The medical treatment and rehabilitation of individuals who have suffered a stroke can be costly. This includes hospital expenses, medical consultations, medications, and rehabilitation therapy. Both the public healthcare system and private insurers allocate significant resources to treat stroke patients.

- Productivity Loss: Stroke often causes severe disabilities that can hinder or even prevent an individual from working. This results in a loss of productivity not only for the individual but also for the overall economy. Rehabilitation and vocational support are necessary to help individuals reintegrate into the workforce, but this can be time-consuming and resource intensive.

- <u>Informal Caregivers:</u> Many stroke survivors require constant care, and often, this care is provided by family members or friends. This can lead to a significant burden on informal caregivers, who often need to take time off from work or even resign from their jobs to provide care.

- <u>Long-term Rehabilitation:</u> Stroke rehabilitation can be a lengthy and costly process. This includes physical, occupational, and speech therapies, as well as home adaptations and medical equipment. Access to these services may vary based on location and available resources.

- <u>Impact on Quality of Life</u>: Individuals who have suffered a stroke often face significant challenges in terms of mobility, communication, and activities of daily living. This can have a negative impact on their quality of life and societal participation.

In 2020, the average economic impact per patient who suffered a stroke in Spain was 41,950 $ (39,780 €). This fact places Spain among the European countries where patients experience one of the highest economic expenses related to stroke sequelae (Figure 3).

In summary, stroke has a substantial socioeconomic impact, reflected in medical costs, productivity loss, caregiver burden, and a diminished quality of life for affected individuals. Effective medical care, rehabilitation, and prevention measures are essential to address this challenge and reduce its impact on society and the economy.

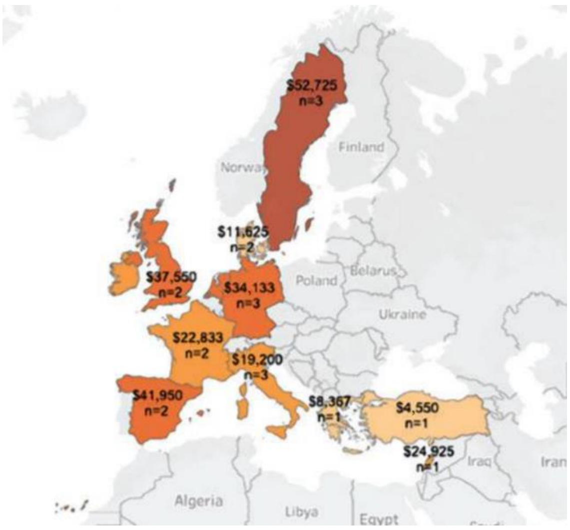

Figure 3. Europe map depicting 2020 per-patient costs in USD, with colors indicating varying values based on analyzed study data. 'n' represents the number of studies considered (6).

1.1.3. PATHOPHYSIOLOGY

Stroke is a complex neurological event with profound clinical implications. Understanding the underlying pathophysiology is pivotal in the development of effective management and prevention strategies. The intricate mechanisms involved in the two primary types of strokes (ischemic and hemorrhagic) is described in this section.

Ischemic Stroke (8)**:**

It constitutes the majority of stroke cases, arising from the occlusion of cerebral arteries, disrupting cerebral blood flow. Two primary mechanisms are responsible for this occlusion:

- ➔ Cerebral Arterial Thrombosis (9): Thrombotic strokes occur when an intracranial artery's lumen narrows due to atherosclerosis, ultimately leading to thrombus formation. This arterial obstruction deprives neurons of vital oxygen and nutrients.

- ➔ Cerebral Arterial Embolism (10): Embolic strokes result from emboli, usually thrombi originating elsewhere in the body, dislodging and traveling to the cerebral vasculature. These emboli impede blood flow in distal cerebral arteries.

In both scenarios, the ischemic cascade, involving excitotoxicity, inflammation, and apoptosis, leads to neuronal injury and death. Reperfusion strategies such as thrombolytic therapy aim to restore blood flow timely and mitigate further damage.

Hemorrhagic Stroke (11)**:**

Though less frequent, it carries a higher mortality rate. They occur when blood vessels within the brain rupture, leading to extravasation of blood into the neural tissue. (12): Intracerebral hemorrhage results from the rupture of small penetrating arteries, often associated with hypertension or amyloid encephalopathy. The extravasated blood increases intracranial pressure, compressing and damaging adjacent brain tissue.

Both ischemic and hemorrhagic strokes can lead to significant neurological deficits, necessitating prompt medical intervention. Understanding the intricate pathophysiological processes underlying each stroke subtype is essential for tailoring effective treatment strategies and enhancing stroke prevention initiatives.

1.1.4. CLINICAL SIGNS AND SYMPTOMS

Patients with stroke are currently categorized into four groups based on their presenting symptoms and signs. It is imperative to underscore that the classification relies on the clinical pattern observed at the time of the most severe symptoms following the initial cerebrovascular event, rather than the pattern seen during our examination, as both typically coincide (13):

Lacunar Infarctions (LACI): These patients currently exhibit several characterized syndromic presentations such as pure motor stroke, pure sensory stroke, sensorimotor stroke, or ataxic hemiparesis.

- ❖ **Total Anterior Circulation Infarctions (TACI):** Patients with TACI currently present a combination of higher cerebral dysfunctions such as dysphasia, dyscalculia, visuospatial disorders, homonymous visual field defects, and ipsilateral motor and/or sensory deficits affecting at least two areas among the face, arm, and leg.

- ❖ **Partial Anterior Circulation Infarctions (PACI):** Patients in this group currently manifest only two of the three components observed in TACI syndrome or exclusively exhibit higher cerebral dysfunction. Some also presently display motor/sensory deficits that are more limited than those classified as LACI, such as confinement to one limb or involvement of only the face and hand but not the entire arm.

- ❖ **Posterior Circulation Infarctions (POCI):** These patients may display motor/sensory deficits that are more likely to be crossed (ipsilateral cranial nerve deficits and contralateral long tract signs) or isolated homonymous visual field defects.

The next algorithm (Figure 4) was constructed based on the OCSP (Oxfordshire Community Stroke Project) classification, as a compilation of neurological examination findings, to make this classification readily comprehensible (14).

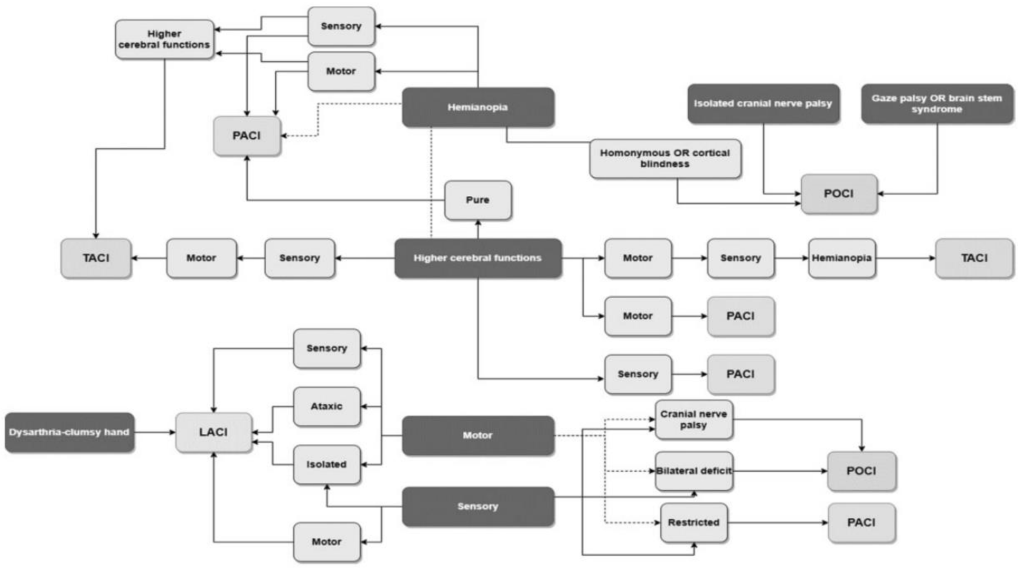

Figure 4. Classification of Stroke according to the Oxfordshire Community Stroke Project Stroke Classification (14).

1.1.5. DIAGNOSIS

The diagnosis of a CVA, is a critical process involving swift and precise identification of symptoms and confirmation of the stroke subtype to facilitate appropriate treatment. Presented below is a succinct outline of the diagnostic procedure:

1. **Initial Clinical Assessment:** The initial step involves a comprehensive medical evaluation. The physician gathers information concerning the patient's symptoms, medical history, and any vascular risk factors, such as hypertension, diabetes, or family history of stroke. Prompt initiation of this assessment is imperative, as time is of the essence in stroke management.

2. **Assessment Scales:** Standardized assessment scales, such as the Glasgow Coma Scale and the NIHSS, are employed to gauge symptom severity and ascertain the extent of cerebral damage.

3. **Brain Imaging:** Neuroimaging is pivotal for confirming the diagnosis of stroke. Common imaging modalities encompass CT and MRI. These images permit clinicians to visualize the brain and discern areas of injury, hemorrhage, or vascular occlusion (Figure 5).

4. **Laboratory Studies**: Blood tests may be conducted to verify glucose levels, coalgulation parameters, and other markers aiding in the determination of the underlying cause of the stroke and those conditioning the prompt initiation of revascularization treatment.

5. **Angiography**: In select cases, cerebral angiography may be performed to evaluate cerebral blood vessels and identify stenosis or occlusion.

6. **Electrocardiogram (ECG):** An ECG is executed to assess cardiac rhythm and exclude atrial fibrillation or other cardiac anomalies that might elevate the risk of stroke.

7. **Hypercoagulation Evaluation:** Coagulation tests are performed to rule out coagulopathy as a causative factor for the stroke.

The precise diagnosis of the stroke subtype (ischemic or hemorrhagic) is paramount, as it dictates treatment options.

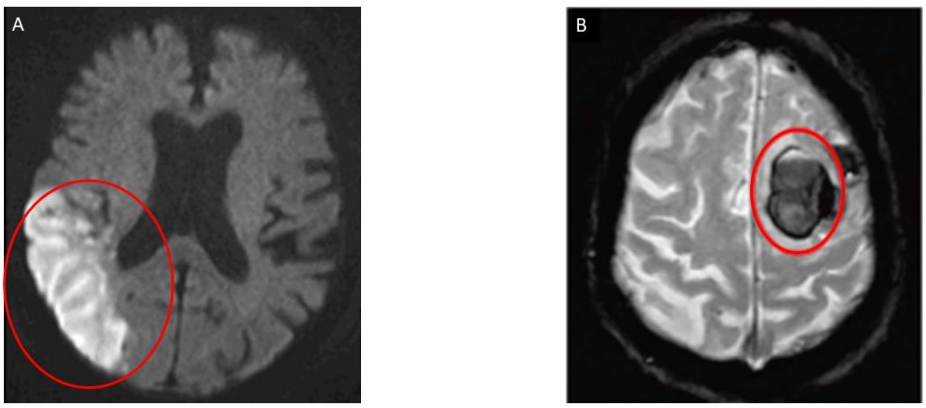

Figure 5. Dual-contrast magnetic resonance imaging (MRI) depicting two distinct types of stroke. A. Large right parietooccipital ischemic stroke. B. Sizeable left frontal hemorrhage (15).

1.1.6. ACUTE PHASE TREATMENT

1.1.6.1. PHARMACOLOGICAL AND INTERVENTIONAL

In recent years, there have been significant developments in the management of acute stroke, resulting in an increased number of patients receiving interventions aimed at minimizing long-term disability. A pivotal advancement in this field has been the establishment of well-organized regional stroke care systems, which are capable of promptly identifying stroke patients in the field. These systems leverage decision support tools to efficiently transport patients to specialized facilities equipped to deliver cutting-edge care tailored to their specific conditions. This comprehensive approach encompasses the essential clinical and imaging assessments, with the results being reviewed by expert clinicians trained in assessing eligibility for expedited administration of intravenous thrombolytic therapy and endovascular thrombectomy. Furthermore, the devices employed for endovascular thrombectomy are continuously enhanced, while the procedural techniques and subacute patient management are continually refined. The onset of acute stroke serves as the opportune moment to initiate acute interventions designed to prevent further strokes in this high-risk population. The appropriate application of available treatments is of paramount importance in optimizing outcomes for stroke patients (Figure 6) (16).

Hemorrhagic and ischemic stroke, urgent treatment should be carried out in a stroke unit due to the potential risk of serious complications.

Regarding hemorrhagic stroke, adequate control of blood pressure, early reversal of anticoagulation, and prevention and treatment of bleeding are essential. Large or life-threatening cerebellar hematomas must be evacuated urgently. However, the indication for surgery is controversial and should be individualized in supratentorial hemorrhage, since it does not improve the functional prognosis. Minimally invasive surgery is offering exciting results and is probably the way to go in the near future (17).

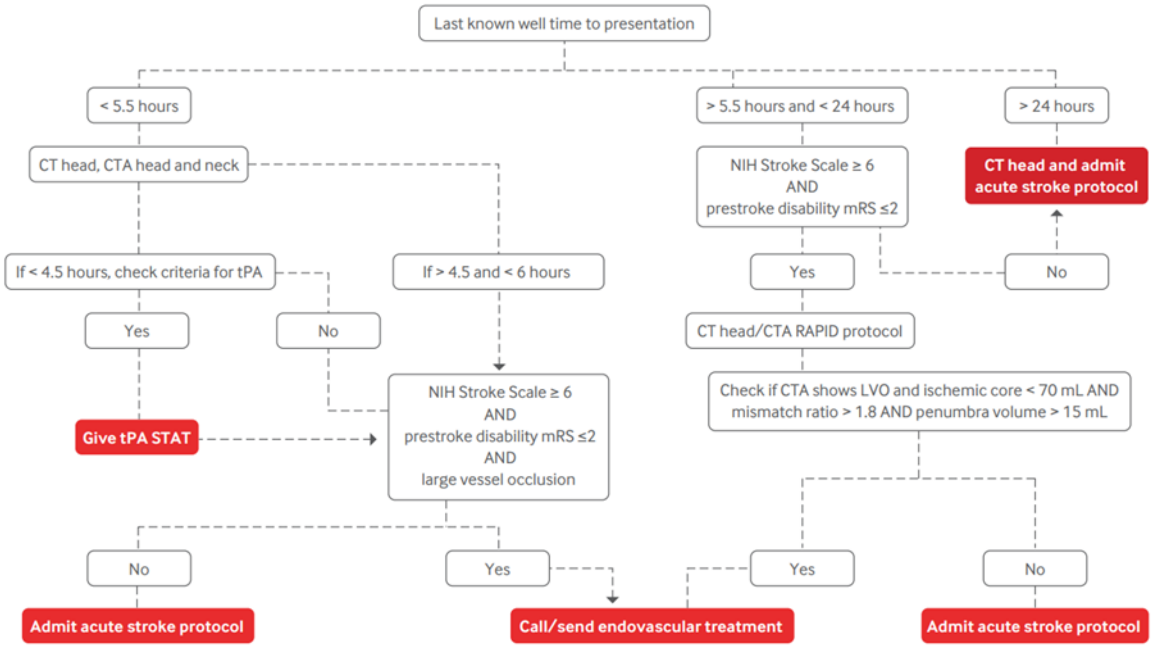

Figure 6. A systematic approach for diagnosing and treating acute strokes (16).

Regarding the healthcare network for Stroke Units or Teams, in Spain the autonomous communities report having 133 Stroke Teams, 71 Stroke Units, and 69 reference hospitals. In the case of the Autonomous Community of Madrid, the Stroke Registry indicates a 40% increase in the activation of treatment codes (within a time window of <4.5 hours) during the period from 2014 to 2019 (5).

1.1.7. SEQUELAE

On the other hand, the classification known as The American Heart Association Stroke Outcome Classification proposed by the AHA categorizes neurological deficits that occur in a patient after a stroke into six areas: motor, sensory, language, visual, cognitive, and emotional (18).

We will address the consequences by focusing on two subgroups: cognitive-emotional and motor-sensory.

1.1.7.1. COGNITIVE-EMOTIONAL

A stroke can result in cognitive impairments, including memory problems, attention difficulties, and challenges in problem-solving and decision-making. Language issues, changes in personality, and difficulties in spatial and temporal orientation are also common (19).

Post-Stroke Depression (PSD) is a common complication following a stroke, impacting patients' mental, linguistic, and cognitive functions. Cognitive impairments are prevalent after strokes, particularly affecting language, attention, comprehension, calculation, and memory, with severity increasing with age. Frontal lobe lesions are an independent risk factor for cognitive impairment, and larger lesions in specific brain regions are associated with a higher incidence of dementia. Although cognitive impairment incidence is similar among cerebral infarction types, TACI patients experience more severe cognitive decline. (20).

1.1.7.2. MOTOR-SENSORY

The most common physical consequence of a stroke is hemiplegia, with approximately 90% of stroke survivors experiencing hemiparesis (21). Hemiparesis results from a unilateral injury to the pyramidal tract, leading to the loss of mobility in one side of the body. This paralysis occurs on the opposite side of the brain lesion due to the crossing of fibers in the affected pyramidal system. Hemiplegia strictly refers to complete paralysis, while incomplete paralysis or paresis is termed hemiparesia . Initially, there's a phase of muscle hypotonia in the affected side, known as flaccid paralysis, which later typically transitions to spastic paralysis or paresis (22,23).

Involvement of the pyramidal system, combined with para-pyramidal pathways, results in the upper motor neuron (UMN) syndrome, characterized by both positive and negative signs. Positive signs include spasticity, co-contraction, flexor and extensor spasms, clonus, heightened tendon reflexes, associated reactions, and a positive Babinski sign. Negative signs encompass weakness, loss of dexterity, and fatigue (24,25). The interplay of these signs can lead to structural changes in tissues, causing stiffness, contractures, fibrosis, and atrophy (26).

Spasticity, a common issue in stroke patients, is considered part of the UMN lesion. Its definition lacks consensus due to its complexity. One widely accepted definition, proposed by Lance et al. (27), characterizes it as a motor disorder marked by a velocity-dependent increase in the tonic stretch reflex with exaggerated tendon reflexes due to hyperexcitability of the myotatic reflex.. Clinically, spasticity is characterized by the typical involvement of muscle groups, predominantly antigravity muscles, an enhanced response to muscle stretching, and heightened tendon reflexes.

It's estimated that spasticity affects one in four stroke survivors and is present in 19% of patients after 3 months, with a prevalence ranging from 25% to 43% during the first year post-stroke (28,29). Furthermore, healthcare costs are four times higher when this motor disorder is present, making it a disabling condition that can cause walking difficulties (30), pain, loss of mine motor skills (31) and reduce the patient's quality of life.

Concerning sensory area, patients can articulate feelings of numbness, tingling, or changes in tactile and kinesthetic sensation. This encompasses more intricate perceptual disruptions such as astereognosis, agraphia, or simultaneous double stimulus extinction, among other manifestations (32). Somesthesis stands as one of the most significant sources of sensory information for the motor system (33). The somatosensory system effectively manages and conveys various modes of somatic sensations, including touch, pain, temperature, and proprioception. The re-establishment of sensory processing and sensorimotor interactions in the motor system compromised by a stroke appears to be pivotal for enhancing motor function (34).

Following a stroke, most patients (69-89%) experience impairments in the upper limb (UL), with hemiparesis being the most common complication (35). This significantly impacts limb strength, manual dexterity, sensory capacity, and limb functionality, contributing to disability in these patients (36). The hand is particularly susceptible, and essential functions such as gripping and manipulating objects are impaired in most cases, with complete recovery being rare. Hemiparesis often leads to a typical flexion pattern in the upper limb, resulting in deformities and mobility challenges. Additionally, issues like intentional underuse of the affected limb can exacerbate these complications (37). Collectively, these alterations exert a substantial impact on the functionality and independence of patients following a stroke.

1.1.8. PROGNOSIS

One comprehensive study (38) delves into the critical issue of prognostic factors in stroke, shedding light on key determinants of stroke outcomes. Advanced age emerged as a significant predictor of unfavorable prognosis, underscoring the intricate interplay between age-related comorbidities and neuroprotective mechanisms. Cardiovascular factors, especially cardioembolic infarcts, were found to be associated with poorer short- and long-term outcomes, emphasizing the need for tailored care strategies. Furthermore, the lateralization of infarcts, particularly right hemisphere involvement, was linked to higher rates of unfavorable outcomes due to autonomic dysregulation (39).

Exploring the intricacies of prognostic factors in stroke patients, several studies (40–42) delves into the evolution of deficits based on the severity of impairment in various organic functions. However, it's noted that these findings remain somewhat subjective due to the challenge of comparing data across different studies, primarily due to the diverse patient populations involved. Creating study groups with identical characteristics proves unfeasible due to the inherent clinical heterogeneity in stroke cases.

The salient points related to different functional aspects are as follows:

- Language: Language capabilities display a potential for ongoing improvement, possibly extending to a year and a half or even two years post-stroke, warranting prolonged monitoring. Initial severity of language impairment emerges as the most reliable prognostic factor. Other variables of significance encompass lesion localization, size, aphasia subtype, and the patient's pre-stroke condition (43).

- Balance: While balance function can continue to improve for up to two years, the rate of improvement diminishes over time. Poor trunk control in a seated position stands out as an adverse prognostic factor (44).

- Ambulation: The ability to walk is a pivotal function, and its potential recovery hinges on a range of factors, including motor deficits, sensory deficits, visual impairments, and the patient's age. The Motor Index value in the third week of recovery serves as a predictive indicator for the same index at the 6th month. Specifically, if a patient achieves a score of 20% on the Trunk Control Test during the third week, it does not predict a good recovery of walking ability before the 3rd month (44).

- Independence: Indicators of a less favorable functional prognosis include low initial scores on the Barthel and Functional Independence Measure (FIM) scales. An estimate of independence in activities of daily living (ADLs) is offered based on the level of dependency prior to the stroke and the initial Barthel scores. It suggests that it may be feasible for patients to return home if their initial Barthel score is greater than 20%-49%. A Barthel score of 60% implies that the patient may have a shorter hospital stay and might require assistive devices, while a Barthel score of 80% suggests that minimal assistance may be needed (45).

  Regarding the FIM scale, its results may correlate with the expected level of mobility, and a score of 72 makes it easier for a patient to return home (45).

- Cognitive Impairment: Post-stroke depression and anxiety have predictive value in terms of worse recovery and aligns with the results of the Functional Independence Measure (FIM) (46).

- Visual Field Deficits: Impaired visual fields can hinder the progress of rehabilitation programs (47).

- Motor Function: Severe deficits observed at the 3-week mark tend to persist at the 6-month point. Recovery in lower limb function generally outpaces that in the upper limb. Early resurgence of proximal movements in the upper limb within the first 4 weeks doesn't correlate with improved functional recovery. In contrast, an early resurgence in distal function, especially if voluntary manual grip is regained in the first month, holds the potential to predict rudimentary functional improvement by the 5th-6th month. It's important to be aware of the percentages recorded in Western countries at 6 months post-stroke: more than 60% may have non-functional hand for Activities of Daily Living (ADLs), and approximately 20-25% may be unable to walk without assistance (48–50).

1.1.9 REHABILITATION TREATMENT OF MOTOR SEQUELAE

The stages of recovery following a stroke are divided into: Hyperacute (0-24 hours after stroke), acute (1-7 days after stroke), early subacute (7 days to 3 months after stroke), late subacute (3-6 months after stroke), and chronic (6 months or more after stroke) (51). Spontaneous recovery after a stroke begins with complex neurochemical processes triggered by brain injury. Brain injury mechanisms include energy failure, excitotoxicity, oxidative stress, and cell death, starting within minutes and continuing for days, even if blood flow is restored. They result in permanent damage to brain cells and the surrounding area, affecting blood-brain barrier integrity and connected brain regions. Recovery is less understood but involves factors like collateral blood vessels compensating for reduced blood flow, enhanced neuronal plasticity in nearby areas, and peripheral effects like muscle loss and stiffness (Figure 7).

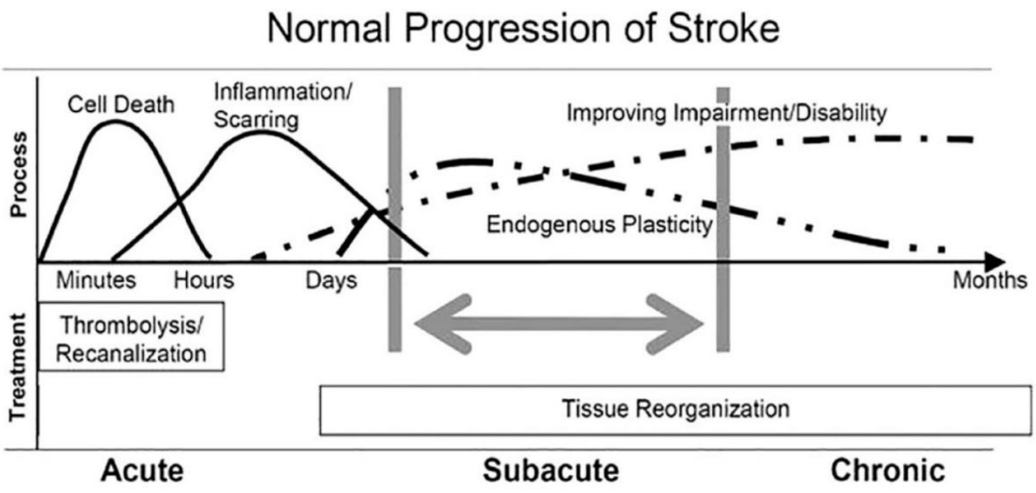

Figure 7. Understanding Stroke Aftereffects: Patterns, Mechanisms, and Therapeutic Prospects (52).

This recovery tends to plateau within about 10 weeks after a stroke (Figure 8), and current evidence doesn't clearly show how rehabilitation interacts with these mechanisms, with improvements often being adaptive as patients learn to optimize limb usage for specific tasks (52).

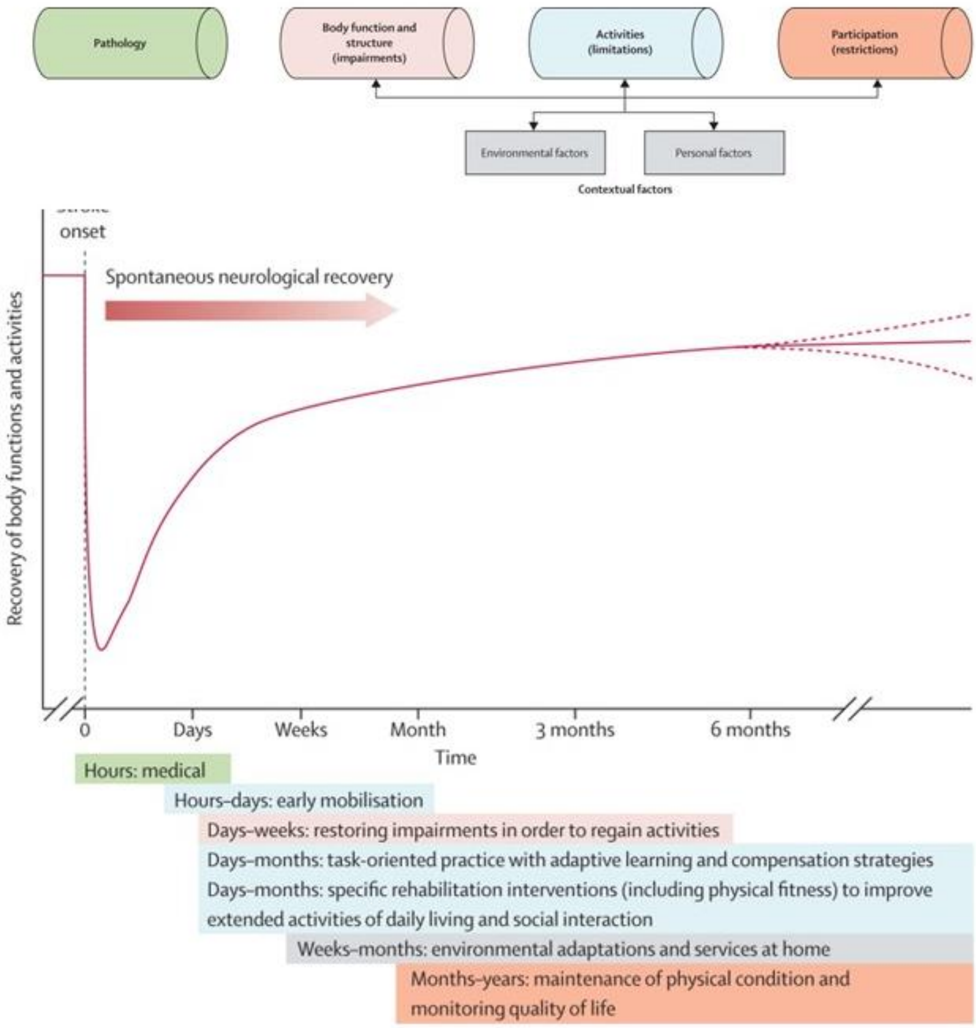

Figure 8. A potential scenario depicting the recovery process following a stroke, including the timing of intervention strategies (53).

The multidisciplinary approach in addressing motor sequelae post-stroke is of paramount importance, involving a collaborative effort among various healthcare professionals. This approach customizes interventions to individual needs, promoting early mobilization, functional recovery, and overall quality of life enhancement. It extends across the continuum of care, ensuring ongoing support and adaptive strategies to optimize motor function and community reintegration, ultimately aiming to restore independence and improve the well-being of stroke survivors (53).

In the next section we will focus specifically on the rehabilitation of the upper limb after stroke.

1.1.9.1 THERAPEUTIC APPROACH TO CHRONIC UPPER LIMB MOTOR SEQUELAE

Regarding the treatment of upper limb impairment in the chronic stage, we present the techniques included in conventional intervention therapies and complementary interventions:

➔ Conventional Rehabilitation: They can be broadly categorized into four main groups:

- *Compensation Techniques:* They aim to enhance independence and function in daily activities. Despite reservations, such as those held by the Bobath method, these techniques can be a valid alternative to improve function in cases of severe motor deficits and an unfavorable recovery prognosis, especially once the patient's motor recovery process has stabilized (54).

- *Facilitation Techniques:* Facilitation techniques emerged in the 1940s with the aim of improving the quality of movement on the affected side in neuromuscular conditions. Despite not demonstrating superiority over other therapies, these techniques, such as the Bobath method, the Brunnstrom method, and Proprioceptive Neuromuscular Facilitation (PNF), continue to be widely used. The Bobath method, for example, is based on clinical observation and aims to normalize muscle tone and improve motor activity. The Brunnstrom method uses afferent stimuli to initiate movement, while PNF focuses on stimulating the nervous system through peripheral stimuli to increase muscle strength and coordination. Although these techniques have a neurophysiological approach, recent research has questioned their superiority compared to other alternatives (55–57).

- *Task-Specific Motor Relearning:* It focuses on teaching stroke patients effective strategies to perform specific functional movements, emphasizing their active participation in the learning process. This method involves clear verbal instructions, visual demonstrations, manual guidance, positive reinforcement, and repetitive practice (58).

- Cognitive Therapeutic Exercise: refers to a rehabilitation technique for the upper limb after a stroke, combining physical exercises to improve arm and hand function with cognitive aspects such as attention, memory, and problem-solving (59).

➔ Complementary techniques to conventional ones:

- Muscle strengthening exercises (50).
- Electrotherapy: High-frequency transcutaneous electrical nerve stimulation (54), Neuromuscular Electrical Stimulation (NMES) (52)
- Non-Invasive Brain Stimulation: Repetitive transcranial magnetic stimulation (rTMS) (41) and Transcranial direct current stimulation (tDCS) (55).
- New technologies applied to neurorehabilitation: Virtual reality interventions (57), Robotic-assisted training (58) and Neurofeedback (60).
- Sensorimotor and Mental Simulation Therapies: Mirror therapy (52) and Mental practice coupled with motor imagery (MI) (53).
- Constraint-induced movement therapy (51).
- Botulinum toxin treatment (56).

One study (52) reviewed national clinical practice guidelines for stroke rehabilitation, focusing on motor rehabilitation interventions, and included guidelines in English and Dutch. Five high-quality guidelines published between 2016 and 2022 were examined, each with varying criteria for "strong" recommendations. The techniques with strong evidence for addressing motor sequelae after a stroke in the chronic stage include traditional or modified constraint-induced movement therapy ((m)CIMT), mental practice, motor imagery, robot-assisted movement therapy, and NMES, all as an adjunct to conventional therapy (52).

It is worth noting that the severity of the initial motor deficit can exert an influence on the feasibility and effectiveness of specific neurorehabilitation approaches, particularly evident in muscle strengthening exercises, constraint-induced movement therapy, and virtual reality interfaces (61).

When treating spasticity, several of these neurorehabilitation approaches have been identified as potential modulators, including botulinum toxin, rTMS, high-frequency transcutaneous electrical nerve stimulation, and tDCS.

However, it is crucial to differentiate between approaches that impact spasticity and those effective in terms of upper limb (UL) motor outcomes (strength, manual dexterity and sensation) but do not substantially affect spasticity. In this category, there is muscle strengthening exercises, neuromuscular electrical stimulation, mirror therapy, constraint-induced movement therapy, and virtual reality (VR) (61).

Regarding UL functionality, a systematic review (61) underscores the positive influence of goal-specific sensorimotor training and task-oriented training on functional recovery after stroke. However, it suggests that while improvements in motor impairment are attainable through various interventions, these improvements do not always translate into enhanced motor abilities in daily activities.

1.1.9.2. LIMITATIONS OF UPPER LIMB REHABILITATIVE TREATMENT IN THE CHRONIC PHASE

Most motor recovery occurs in the first 3 months after a stroke (Figure 8). The neurobiological mechanisms of recovery during the early subacute stage are complex and are still being elucidated. In general, a stroke induces a cascade of effects on gene expression, cellular function, and the structure of surviving tissues, most of which promote recovery. These endogenous mechanisms are broad and most active early after the stroke, and they are largely responsible for the recovery of motor function (51).

However, the chronic stage presents different challenges that lack of effective approaches in most cases. Chronic non-use of the paretic upper limb, general physical deterioration, and limitations in therapeutic effort can influence baseline measures of impairment and capacity. Therefore, the benefits of interventions in the chronic stage may be related to reconditioning that helps patients return to their best functional state rather than specific neurophysiological effects. These challenges make it important to continue researching in these phases.(62–64).

1.2. NON-INVASIVE BRAIN STIMULATION

Neuromodulation encompasses a broad range of invasive and non-invasive techniques aimed at altering the activity or excitability of neurons (65). The therapeutic use of neuromodulation techniques is based on the following scientific findings (66):

- Neuroplastic changes can occur in the human nervous system, which may be associated with altered functional outcomes and/or pathological clinical conditions (67).
- Different neuromodulatory approaches can induce neuroplasticity as they allow for changes in neuronal activity and connectivity, and, therefore, can be used to reverse or prevent maladaptive neuroplastic changes (67).

- It has been demonstrated that promoting adaptive neuroplastic changes and reversing maladaptive changes are associated with functional improvement (66).

Neuromodulation techniques for the nervous system can be classified according to two different criteria (68):

-Location within the nervous system where the application is performed: Peripheral Stimulation or Central Stimulation.

-Invasive or non-invasive application to neural tissue: Non-invasive Neuromodulation (Transcutaneous Electrical Nerve Stimulation, Transcranial Magnetic Stimulation, Transcranial Direct Current Stimulation, and Transcranial Alternating Current Stimulation) and Invasive Neuromodulation (Perutaneous Electrical Nerve Stimulation, Peripheral Nerve Stimulation, Occipital Nerve Stimulation, Sphenopalatine Ganglion Stimulation, Vagus Nerve Stimulation, Deep Brain Stimulation, Spinal Cord Stimulation, and Motor Cortex Stimulation).

1.2.1. TRANSCRANIAL MAGNETIC STIMULATION

Transcranial Magnetic Stimulation (TMS) is a non-invasive, focal, and painless brain neuromodulation technique that follows the fundamental principles of electromagnetic induction. In this technique, an electric current in the stimulation coil generates a magnetic field, which in turn induces a new electric current in nearby conductors. When the stimulation coil is placed tangentially on the skull, it generates a perpendicular magnetic field that, in turn, induces an intracranial electric current parallel to the coil and in the opposite direction, thereby focally stimulating the cerebral cortex (69).

TMS can be classified based on how the stimulation is administered (70):

-Single-Pulse TMS: Involves the administration of a single magnetic pulse to the cerebral cortex at a specific moment. This type of application is often used to investigate specific brain functions in healthy individuals and patients with neurological and psychiatric disorders (71).

- **Paired-Pulse TMS:** Involves the administration of two magnetic pulses with a precise time interval between them. The first pulse is used to "prepare" the cerebral cortex, and the second pulse enhances the effectiveness of stimulation. This form of TMS has been utilized to investigate functional connectivity between different brain areas and is valuable in the research of neurological and psychiatric disorders (72).

-**<u>Repetitive TMS (rTMS):</u>** This form of TMS delivers a series of repetitive magnetic pulses at a specific frequency. rTMS involves the administration of repetitive magnetic pulses at a specific frequency over an extended period of time, which can alter neuronal activity in the brain (73).

1.2.1.1. NEUROPHYSIOLOGICAL EFFECTS OF TMS

Since the first application of TMS in humans in 1985 (74), there has been rapid growth in the study of this field; however, the basic principles remain the same. As previously mentioned, the coil is placed tangentially to the scalp and induces a magnetic field perpendicular to its plane, which penetrates the skin, scalp, and skull, reaching the brain. Within the brain, the pulse of the magnetic field induces an electric current perpendicular to the magnetic field and, therefore, parallel to the coil's plane.

The currents are capable of eliciting action potentials in the neurons of the stimulated cerebral region (75). Epidural recordings in patients with implanted spinal electrodes have shed light on this process. Gradually increasing the intensity of single-pulse TMS applied to the human M1 leads to a growing number of descending corticospinal volleys recorded at the cervical spinal cord level. These volleys comprise the direct-wave (D-wave) and indirect-waves (I-waves), generated through direct and indirect corticospinal excitation. Various factors, including stimulation intensity and coil shape, affect the recruitment of these volleys and require standardization for meaningful comparisons. Glutamate release in cortico-motoneuronal synapses induced by these volleys leads to postsynaptic depolarization, and when the cumulative strength surpasses the firing threshold, it triggers action potentials in spinal motoneurons, resulting in Motor Evoked Potentials (MEPs). The efficacy of cortico-motoneuronal excitation depends on both external and internal factors, and enhancing this excitation, such as through increased stimulus intensity or muscle contraction, leads to MEP amplitude augmentation and shorter latency. TMS-induced corticomuscular excitation exhibits more temporal dispersion compared to compound muscle action potentials (CMAPs) obtained through electrical nerve stimulation, contributing to differences in MEP characteristics (76,77) (Figure 9).

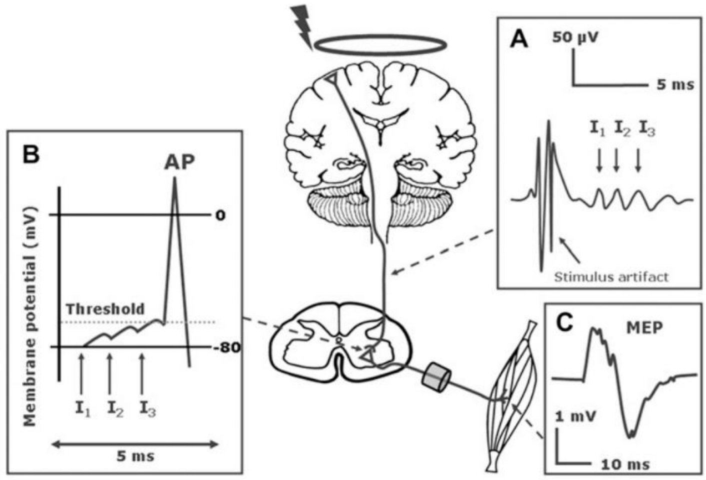

Fig. I.

Figure 9. The neurophysiological foundation of the motor evoked potential (MEP) can be understood through two key processes. A. TMS activates corticospinal neurons, primarily through the involvement of late I-wave. B. Temporal and spatial summation of neural activity at the synapses between the cortex and motoneurons. This cumulative process ultimately leads to the generation of the motor evoked potential (MEP) (76).

Di Lazzaro et al. (78) conducted a study to assess whether the primary features of the activity evoked by repetitive and single-pulse and paired-pulse TMS can be explained by the interaction of induced currents in the brain with key anatomical features of a simple cortical circuit. This circuit consisted of excitatory pyramidal neurons in layers II and III, large pyramidal neurons in layer V, and GABAergic inhibitory cells. According to their findings, this circuit represents the minimal architecture necessary to capture the most essential input-output operations of the neocortex. The interaction between the induced currents in the brain and this simplified model of cortical circuits could explain the characteristics and nature of the repetitive discharge evoked by TMS. The integrative properties of the circuit also provide a robust framework for interpreting changes in cortical output produced by paired pulse and repetitive TMS.

1.2.1.2. REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION

rTMS differs from single-pulse and paired-pulse TMS in that magnetic stimulation is applied repetitively and with specific patterns, allowing for more precise and long-lasting modulation of neuronal activity.

When repetitive stimuli are applied, we will address two neurophysiological effects that form the basis for the therapeutic use of this type of stimulation (79):

-Long-Term Potentiation (LTP) is a phenomenon that refers to the increase in synaptic strength that occurs when a synapse is repetitively stimulated at a high frequency. This is achieved by activating NMDA receptors on the cell membrane of the postsynaptic neuron, triggering a series of molecular events that enhance the synapse's sensitivity to neuronal input and, consequently, increase its strength (80).

- Long-Term Depression (LTD) is a phenomenon that refers to the decrease in synaptic strength that occurs when a synapse is repetitively stimulated at a low frequency. In this case, the activation of NMDA receptors is insufficient to produce LTP, and instead, metabotropic glutamate receptors are activated, triggering a series of molecular events that reduce the strength of the synapse (80).

It's worth noting that rTMS has been used in experimental studies to induce changes in long-term synaptic plasticity in the brain (81). For instance, high-frequency rTMS has been used to induce LTP in specific areas of the brain, while low-frequency rTMS has been employed to induce LTD in specific brain regions (82). These findings can be valuable for the development of therapeutic treatments for various neurological conditions believed to involve changes in long-term synaptic plasticity (81).

1.2.1.3. PARAMETERS OF rTMS

There are several parameters that can influence non-invasive brain neuromodulation protocols with TMS. Some of the most important ones include (83,84):

- **Frequency:** it is a key parameter in most non-invasive brain neuromodulation protocols. Frequency refers to the number of magnetic pulses delivered per second and is measured in hertz (Hz). There are two main frequencies commonly used in TMS (85):

- *Low-Frequency rTMS (LF-rTMS):* Generally defined as a stimulation frequency equal to or less than 1 Hz. It has been demonstrated that this frequency can reduce neuronal excitability in the stimulated brain area, thus producing an LTD effect.

- *High-Frequency rTMS (HF-rTMS):* Defined as a stimulation frequency greater than 1 Hz, although values exceeding 5 Hz are often used. It has been shown that this frequency increases neuronal excitability in the stimulated brain area, resulting in a LTP effect.

There is a specific high frequency rTMS protocol called Theta Burst Stimulation. This technique involves delivering pulses in bursts at a frequency of 50 Hz. There are two main types of Theta Burst used in TMS (86):

- *Continuous Theta Burst (cTBS):* In this type of stimulation, bursts of magnetic pulses are applied at a frequency of 50 Hz continuously for a period of 40-60 seconds. This modality is associated with LTD processes (87).

- *Intermittent Theta Burst (iTBS):* In this type of stimulation, bursts of magnetic pulses are applied in groups of three pulses separated by 200 ms intervals. Each burst group is repeated at a frequency of 5 Hz for 2 seconds, followed by a rest period of 8 seconds, and this cycle is repeated for a total duration of 190 seconds. In this case, iTBS is associated with LTP processes (88).

- **Intensity:** refers to the amount of magnetic energy delivered in each pulse and is measured as a percentage of the maximum intensity that can be delivered. In TMS, intensity is adjusted to produce the desired level of neuronal stimulation in the target brain area. Typically, two types of intensity are used (89):

- *Subthreshold Intensity*: refers to a TMS intensity level that is too low to elicit a visible motor response in the stimulated brain area.

- *Suprathreshold Intensity* refers to a TMS intensity that is high enough to evoke a visible motor response in the stimulated brain area. This intensity is often used to locate the motor cortex and adjust the positioning of the TMS coil.

It's important to highlight that the intensity of TMS is individually adjusted for each patient and determined based on patient safety, tolerance, and the objectives of the stimulation. Additionally, it's crucial to establish the minimum intensity required for the target brain area to generate a response beforehand.

Once this threshold is determined, the intensity for the protocol can be calculated. In the case of motor cortex stimulation, this is referred to as the motor threshold, representing the minimum energy needed to elicit a motor response.

- **Session Duration:** In general, TMS sessions last between 20 and 60 minutes. The exact duration of the session depends on the frequency and number of pulses delivered during each session, as well as the specific therapeutic protocol being used (85).

- **Number of Sessions:** The number of sessions varies depending on the condition being treated, the objective of the stimulation, the protocol used, and the individual patient's response to therapy (85).

- **Stimulation Location:** The location of stimulation is an important factor that can vary depending on the condition being treated. Stimulation can be applied to different regions of the brain (85).

- **Stimulation Coil Type:**

There are several types of coils used in the technique of transcranial magnetic stimulation (TMS). Below, we briefly describe the 4 common types of coils used (89):

- **Figure-of-Eight Coil:** This is the most used coil in TMS. It has an "eight" or figure-eight shape and is used to stimulate specific regions of the brain, such as the dorsolateral prefrontal cortex or the primary motor cortex. These coils have two poles separated by a small distance, allowing for the focalization of magnetic pulses to a specific brain region (90) (Figure 10).

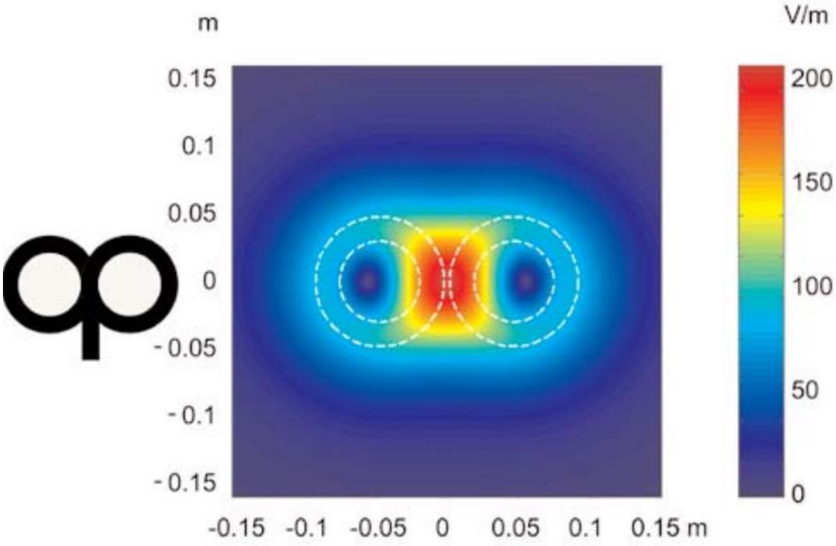

Figure 10. The distribution of induced electric fields by an 8-shaped coil (b) is depicted here. The 8-shaped coil has a diameter of 56 mm for the inner loop, 90 mm for the outer loop (averaging 73 mm) and contains nine turns of copper wire in each wing. The external morphology of each loop is represented by discontinuous white lines superimposed on the induced field representation. The electric field amplitude is calculated for a plane located 20 mm below the coil in a realistic model (dL/dt = 108 As-1) (90).

- **Double Cone Coil:** This is a variant of the figure-of-eight coil, featuring two cones instead of two separated poles. It provides higher focalization than the figure-of-eight coil and is used to stimulate specific brain regions with greater precision.
- **H-Coil:** The H-coil consists of two figure-of-eight coils that intersect at a 90-degree angle, enabling greater focalization and precision in stimulation. The coil's

shape also allows for better adaptation to the patient's skull shape, improving the transmission of magnetic pulses to the brain.

- **Circular Coil:** It has a circular shape and is used to stimulate broader brain regions. The focalization of the circular coil is lower than that of figure-of-eight coils, H-coils, or double cone coils. The focalization of the circular coil is related to the size of the coil and its internal diameter. In general, the larger the coil, the better its ability to stimulate larger brain areas, but the lower its focalization.

The choice of TMS coil depends on the brain area to be stimulated, the depth of stimulation, the stimulation objective, and the patient's tolerance.

It's important to emphasize that TMS parameters can vary depending on the type of pathology and the patient. Therefore, the selection of appropriate parameters should be made in consultation with a qualified medical or healthcare professional experienced in this field.

1.2.1.4. APPLICATIONS OF TMS

TMS is used for various purposes, which can be grouped into diagnostic use, neurophysiological research, and therapeutic applications.

1.2.1.4.1. DIAGNOSIS

The primary use of TMS in clinical neurophysiology is to assess the conduction of the corticospinal pyramidal pathway by recording motor-evoked potentials (MEPs). MEPs are recorded on target muscles using surface electrodes and a bipolar setup (85).

Therefore, when using TMS to record MEPs, the total motor conduction time (TCMT) from the motor cortex to the target muscles can be broken down into two components: (1) central motor conduction time (CMCT), which includes conduction along the pyramidal pathway from the upper cortical motor neurons to the lower motor neurons and the proximal part of the peripheral nerve (up to the intervertebral foramina or stylomastoid foramen), and (2) peripheral motor conduction time (PMCT), which measures the conduction of the peripheral nerve at a distance from the cell body of the lower motor neuron (91).

Furthermore, it's important to emphasize that with single-pulse TMS, the upper cortical motor neurons are generally excited trans-synaptically through intracortical interneurons, unlike high-intensity transcranial electrical stimulation, which directly activates the corticospinal pathway within the motor cortex (85).

Navigated transcranial magnetic stimulation (nTMS) has become increasingly important in preoperative mapping of cortical language and motor functions, particularly in patients with brain tumours. Motor mapping involves delivering single TMS pulses above the RMT to the rolandic region, while language mapping uses brief rTMS trains to induce speech arrest or errors. These nTMS techniques have shown impressive accuracy compared to the gold standard of intraoperative direct cortical stimulation (92). In a study (93) of 733 brain tumor patients, nTMS mapping was found to be generally well-tolerated, with a minority reporting discomfort and pain during the procedure. Notably, despite the high seizure risk in these patients, no seizures were reported during nTMS mapping, indicating its safety for motor and language mapping applications.

1.2.1.4.2. NEUROPHYSIOLOGICAL RESEARCH

Currently, there is a continuous production of scientific literature on the neurophysiological effects that underlie the utility of TMS in addressing various pathologies. To reach this point, it has been necessary to study neurophysiological components that are not used in clinical practice but are employed in research (94).

At this stage, TMS has a specific role in the study of:

a) <u>Cortical Brain Excitability</u>: This refers to the cortex's capacity to respond to stimuli, whether they are external or internal, through changes in electrical activity (95). To assess this measure, the following are often used:

- *Motor Thresholds*: the level of TMS intensity required to produce a measurable motor response in a peripheral muscle, either when the muscle is previously contracted (Active Motor Threshold) or at rest (Resting Motor Threshold) (95).

- *Amplitude of MEPs:* This is another commonly used measure to assess cortical excitability (96).

b) <u>Intracortical Inhibition-Facilitation:</u>

-*Long-Interval Intracortical Inhibition:* This is obtained when two supra-threshold stimuli are applied with intervals between 50 and 200 ms and is believed to reflect neurotransmission mediated by GABA-B receptors (97).

-*The Cortical Silent Period* is also a marker of cortical activity inhibition, presumably mediated by GABA-B receptors, and is obtained when a TMS pulse is delivered during the tonic contraction of the target muscle (98).

-Short-Interval Intracortical Inhibition: A subthreshold conditioning stimulus inhibits an MEP produced by a single suprathreshold pulse at short intervals of 2-3 ms. This marker is associated with GABA-A receptor activity (99).

-*Intracortical Facilitation:* Conversely, longer intervals (7-30 ms) result in facilitation of MEPs. The excitation associated with this marker involves both GABA-A and NMDA receptors (100).

-*Short-Latency Afferent Inhibition:* The combination of electrical stimulation of the median nerve with TMS leads to suppression of the MEP when the stimulation precedes a single pulse applied to the Primary Motor Cortex (M1) at short intervals of 20 to 25 ms. This marker has been primarily associated with cholinergic and GABAergic circuits. (101).

1.2.1.4.3. EFFICACY OF TMS IN MOTOR REHABILITATION POST-STROKE

The therapeutic use of rTMS is a reality, as both the U.S. Food and Drug Administration and the European Medicines Agency, among others, approved the use of rTMS in 2008 for the treatment of treatment-resistant depression and neuropathic pain (102). Regarding depression, there is level A evidence for the antidepressant efficacy of HF-rTMS stimulation over the left dorsolateral prefrontal cortex. Regarding chronic pain, there is also level A evidence for the analgesic efficacy of HF-rTMS stimulation over the contralateral primary motor cortex to the side of pain (103).

Regarding stroke, updated guidelines by expert committees do not endorse any specific protocol, but they emphasize the existing level of evidence in the use of rTMS to treat UL motor sequelae after a stroke. Specifically, they state that there is conclusive evidence that low frequency rTMS over the contralateral motor cortex is effective in motor hand function recovery in the subacute stage. Additionally, there is a likelihood of efficacy in the use of HF-rTMS over the ipsilateral motor cortex for hand motor recovery in the same stage. Lastly, there is a possible efficacy in the use of LF-rTMS over the contralateral motor cortex for hand motor recovery in the chronic stage (103).

In the following Table 1, we found 28 experimental studies until July 2022 where rTMS was employed for the rehabilitation of upper limb motor sequelae in chronic stroke. Out of these 28 articles, 26 utilized rTMS as an adjunct therapy to conventional rehabilitation or complementary techniques. This finding emphasizes the need for further investigation, whether in online or offline paradigms, to explore the complementary effects of rTMS with other innovative upper limb stroke rehabilitation techniques. The combined effects appear to offer greater benefits compared to isolated rTMS interventions (50).

Table 1. rTMS studies to rehabilitate motor sequelae in chronic strokes.

Study	Design	Objective
(104)	Randomized, single-blinded, controlled trial.	To investigate the effect of neuromuscular electrical stimulation (NMES) combined with rTMS on poststroke hemiparetic upper limb rehabilitation.
(88)	Randomized, single-blinded, controlled trial.	To investigate the effects of priming iTBS followed by iTBS + robot-assisted training on poststroke hemiparetic upper limb recovery
(105)	Open-label, crossover, controlled study,	To compare the effects of the Program NEURO (rTMS + Occupational therapy) in patients with hemiparesis after stroke
(106)	Randomized, open label, controlled trial study	To evaluate the efficacy of combined rTMS + BCI, compared to sham rTMS + BCI, on motor recovery after stroke.
(107)	Single-blinded, randomized controlled study.	To assess the efficacy of inhibitory rTMS in healthy hemisphere M1 on upper extremity motor recovery and functional outcomes in chronic ischemic stroke patients.
(108)	Open-label, controlled study.	To compare the effects of navigated rTMS between stroke patients and control subjects.
(109)	Randomized, crossover, single-blinded design	To evaluate changes in cortical excitability and paretic hand function in chronic stroke following three types of primed low frequency rTMS treatments on healthy hemisphere M1.
(110)	Open-label, single-arm study.	To investigate the safety of contralesional M1 rTMS in a selected group of severe chronic stroke patients.
(111)	Randomized, double-blinded, controlled study.	To assess the effects of low frequency rTMS in healthy hemisphere M1 as an adjuvant to functional task practice, to improve upper extremity function.

(112)	Randomized, single-blinded, crossover study.	To determinate the most effective method for combining rTMS and motor training in stroke patients.
(113)	Randomized, double-blinded, controlled trial.	To assess the efficacy of inhibitory rTMS for decreasing upper-limb muscle tone after chronic stroke.
(114)	Randomized, single-blinded, controlled trial.	To analyse the characteristics of responder vs. non responders receiving rTMS to improve hand function after stroke.
(115)	Randomized double-blinded crossover study	To investigate whether multiple sessions of 1-Hz rTMS on healthy hemisphere M1 facilitates the effect of repetitive facilitation exercises on hemiplegic upper-limb function in chronic stroke patients.
(116)	Randomized, single-blinded, controlled, crossover study	To investigate the effect of inhibitory low frequency rTMS applied to the non-lesioned hemisphere on kinematics and coordination of paretic arm reach-to-grasp action.
(117)	Randomized, single-blinded, controlled study.	To investigate the potential for a consecutive suppressive-facilitatory rTMS protocol to improve motor outcomes after chronic stroke.
(118)	Randomized, double-blinded, controlled, study.	To investigate the long-term behavioural and neurophysiologic effects of rTMS on healthy hemisphere M1 combined with physical therapy intervention in chronic stroke patients with mild motor disabilities.
(119)	Semi-randomized, single-blinded, controlled trial.	To explore whether long-lasting clinically important gains can be achieved by adding TBS to a rehabilitation program for the hand.
(120)	Double-blinded, controlled, crossover study.	To determine the effects of M1 TBS and standardized training on upper-limb function of patients with chronic stroke.

(121)	Randomized, double-blinded, study.	To investigate whether bilateral rTMS stimulating injured M1 and inhibiting healthy M1 may improve the paretic hand in patients after stroke.
(122)	Randomized, double-blinded, controlled study.	To investigate whether 1 Hz TMS on healthy hemisphere M1 improve the motor learning of the affected hand in patients after stroke.
(123)	Randomized, double-blinded, controlled study.	To test the potential adjuvant effect of rTMS on motor learning in a group of survivors undergoing constraint-induced therapy (CIT) for upper limb hemiparesis.
(124)	Randomized, single-blinded, controlled study	To evaluate whether five sessions of low frequency rTMS on healthy hemisphere M1 produces improvements in upper limb motor function and whether this approach is safe and its effects durable.
(125)	Pseudorandomized, crossover, controlled study.	To investigate high-frequency rTMS-induced cortical excitability and the associated motor skill acquisition in chronic stroke patients.
(126)	Randomized, double-blinded, controlled study.	To study if 1 Hz rTMS on healthy hemisphere M1 improves motor performance of the affected hand in stroke patients by decreasing the transcallosal inhibition
(127)	Double-blinded, controlled, crossover, study.	To investigate the use of low frequency rTMS on the unaffected hemisphere to decrease interhemispheric inhibition of the lesioned hemisphere and improve motor function in patients after stroke.

Acronyms: rTMS: Repetitive Transcranial Magnetic Stimulation; iTB: Intermittent Theta Burst; M1: Primary Motor Cortex.

Figure 11. rTMS Application in a Patient with Chronic Stroke. Self-generated.

1.2.1.4.4. ADVANTAGES AND LIMITATIONS OF CLINICAL APPLICATION OF rTMS

-Advantages:

- It is a non-invasive and relatively safe technique. It does not require surgery or the introduction of devices into the body. Furthermore, studies have shown that TMS is well-tolerated by patients, with few side effects (92).
- It is a versatile technique that can be used both for assessment and for various potential therapeutic approaches to treat a wide range of neurological and psychiatric conditions (128).
- It can selectively and specifically modulate neuronal activity (129).
- In comparison to pharmacological therapy, TMS typically has fewer side effects (92).

-Limitations:

- Effects are often temporary and may fade after a few weeks or months *(128)*.
- TMS devices are expensive and are not always covered by health insurance *(130)*.
- Patients with metallic implants or intracranial electronic devices near the stimulation site cannot undergo TMS *(92)*.
- Depending on the duration of application, patients may be in uncomfortable cervical-cranial positions *(128)*.

1.2.1.5. SAFETY OF TMS

The safety protocols for TMS are updated by expert committees at relatively short intervals due to the increasing number of studies using TMS. Rossi et al. (92) They have published recently recommendations for the safe and ethical use of TMS in healthy subjects and patients. In this publication, they emphasize the importance of individually assessing the risks and benefits of TMS and recommend its use in research and clinical settings with proper supervision.

Regarding safety, they mention the most common side effects of TMS, such as headaches and muscle contractions, which are typically mild and transient. They also discuss the possibility of more serious adverse effects, such as seizures, but emphasize that these are extremely rare (around 1/30,000 in healthy subjects and approximately 1/9,000 in patients with neuropsychiatric disorders).

Regarding frequency, in the 2021 update, they advise against applying high frequency rTMS at 100% of the subject's motor threshold to avoid seizures. Similarly, they recommend not using frequencies higher than 25Hz, except in the case of Theta Burst stimulation.

In terms of intensity, in the 2021 guideline (92), they recommend working with a maximum intensity of 75% in single pulses and applying rTMS with subthreshold intensities (80-90% of motor threshold) to reduce the risk of seizures.

Finally, the same guideline makes recommendations to minimize the risks associated with the use of rTMS, such as conducting a thorough patient assessment before and after stimulation. It also addresses ethical and regulatory issues surrounding the use of rTMS in research and clinical practice, emphasizing the need for informed consent from participants.

1.2.2. MOTOR IMAGERY

Motor imagery (MI) is a complex cognitive process that involves the ability to mentally evoke the execution of a movement (131). The vividness of MI refers to the intensity with which the subject perceives the mental task through somesthetic, kinesthetic, visual, or other types of sensations (132). Kinesthetic MI vividness involves reliving the movement in terms of motor control, while visual MI vividness pertains to the clarity with which the subject visualizes himself performing the movement. Both experiences are subjective and generated through memory. MI should be consistent with the physical execution of the movement in terms of kinematic, dynamic, temporal, and spatial characteristics. Therefore, MI is a valuable tool in rehabilitation and motor skill learning. (133–135).

A technique closely related to MI is Action Observation (AO). This therapy is an emerging approach to rehabilitation that capitalizes on the involvement of the mirror neuron system in motor learning. It integrates motor-based methods with cognitive strategies designed to enhance motor recovery after a stroke (136) .

1.2.2.1. NEUROPHYSIOLOGICAL EFFECTS OF MOTOR IMAGERY

Several studies have demonstrated that mentally generating images of movements activates the same brain areas as actual movement execution, indicating the biological validity of this technique (51,52). The brain areas associated with voluntary motor control include the primary motor cortex, the medial and lateral premotor cortex, the primary somatosensory cortex, and multisensory association cortices, requiring interaction with subcortical structures such as the basal nuclei and the cerebellum. A meta-analysis published in 2018 (52) demonstrated that mental imagery primarily activates a neural network involving cortical and subcortical areas, such as premotor cortices, the parietal lobe, and the dorsolateral prefrontal cortex (Figure 12). Both mental imagery and actual execution share a network comprising sensorimotor and premotor cortical and subcortical clusters.

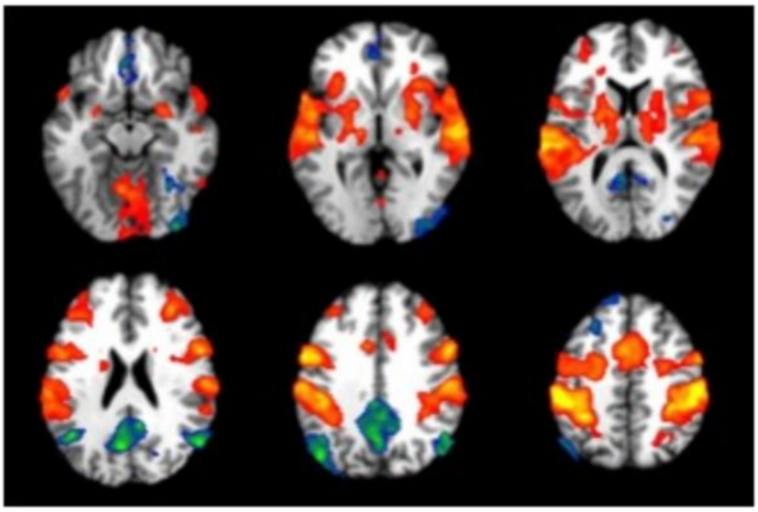

Figure 12. fMRI whole-Brain Activity. A representation of comprehensive brain activation, illustrating regions engaged during motor execution and motor imagery (in yellow and red) in comparison to periods of fixation (in blue and green) (137).

Both, AO and MI, are linked through the activation and observation of mirror neurons. AOT is based on the idea that observing the execution of motor actions activates the same neural networks that would be engaged when performing those actions, potentially facilitating motor recovery (136). In contrast, MI involves the active reproduction of observed movements (138). Both therapies rely on the concept that action observation can influence motor recovery and leverage the mirror neuron system in the brain, which activates both when carrying out an action and when observing others perform it (139). This interconnection between AOT and MI underscores the importance of observation and imitation in motor function rehabilitation and recovery following neurological injury.

1.2.2.2. APPLICATIONS OF MOTOR IMAGERY

There are different uses of motor imagery, including:

-Rehabilitation: Brain or muscular injuries to enhance motor function (140).

-Sports Training: To improve the execution of specific movements and enhance athletes' confidence and concentration (141) .

-Treatment of Psychological Disorders: It is employed as part of the treatment for psychological disorders such as anxiety, post-traumatic stress disorder, and depression (142).

1.2.2.3. MOTOR IMAGERY IN POST-STROKE REHABILITATION

In recent decades, researchers have increasingly examined the potential advantages of utilizing imagery and observation as viable methods to facilitate motor recovery following stroke. Initial investigations suggested that imagery could be a promising therapeutic approach (143). However, more recent research has produced inconsistent findings, with a meta-analysis conducted by Machado et al. (144) indicating that MI may not significantly enhance traditional physical therapy in stroke rehabilitation. This underscores the necessity of identifying the most suitable form of imagery practice for stroke recovery, particularly considering evidence suggesting that stroke survivors may experience compromised imagery abilities. On the contrary, Action observation (AO) therapy has demonstrated more reliable positive results. Notably, studies conducted by Ertelt et al. (145) and Franceschini et al. (146) revealed that a four-week regimen of action observation therapy, which entails observing and subsequently imitating daily activities, resulted in notable enhancements in motor function and limb usage. These improvements were sustained for several months post-intervention. Furthermore, evidence from the realm of sports has shown that exposure to video demonstrations of human actions can augment imagery abilities. Considering the potential effectiveness of both MI and AO in stroke rehabilitation, coupled with indications that AO may lead to improved MI abilities, the combination of AO and MI presents a promising prospect for augmenting motor function in stroke rehabilitation. Early evidence suggests that daily AO and MI therapy over a four-week period can enhance pinch-grip strength following stroke, although additional research is required to corroborate these findings (143).

1.2.2.4. ADVANTAGES AND LIMITATIONS OF CLINICAL APPLICATION OF MOTOR IMAGERY IN STROKE

-<u>Advantages:</u>

- Rehabilitation Without Physical Movement: MI allows patients to mentally practice motor movements without the need for full physical execution. This is beneficial for patients with severe physical limitations following a stroke (147).

- Brain Activation: MI has been shown to activate brain areas similar to those activated during actual movement execution. This can help preserve brain plasticity and promote functional recovery (148) .

- Safety: MI is a safe technique that poses no physical risks to patients, making it suitable for those with additional medical issues (149).

- Cost-Effective: Compared to some other rehabilitation therapies, MI can be more cost-effective and accessible (150).

-<u>Limitations:</u>

- Variable Effectiveness: The effectiveness of MI can vary among patients. Not all patients respond in the same way, and some may not experience significant improvements (151).

- Requires Training: MI requires training and practice to be effective. Patients must learn to perform mental imagery correctly, and this can take time (138).

- Cognitive Limitations: Some patients may have cognitive difficulties that hinder the practice of MI (144).

- Vividness Requirements: MI effectiveness is related to the vividness of mental images. Some patients may struggle to create vivid mental images (152).

- Requires Supervision: MI is often conducted under the supervision of a therapist or healthcare professional, which may require additional resources (153).

1.2.3. NEUROFEEDBACK

Neurofeedback (NFB) involves the measurement and real-time presentation of neural activity through sensory channels to assist individuals in self-regulating the neural processes associated with specific behaviors or pathologies. Both animal and human brains have demonstrated self-regulation using various recording methods, both invasive and non-invasive, and by analyzing different aspects of brain signals, such as frequency patterns, functional connectivity, and spatiotemporal brain activity. NFB training induces neural changes that are pertinent to the specific brain circuit being trained, leading to associated behavioral modifications. These connectivity changes have been observed to persist for varying durations, from hours to months after training, and have been linked to alterations in the structure of gray and white matter in the brain (60).

1.2.3.1. TYPES OF NEUROFEEDBACK

There are seven distinct types of NFB methods used for the treatment of various disorders (154). We are going to classify them into two groups: techniques based on EEG and techniques based on other signals.

Regarding the first of them, NFB technique involves enhancing specific electroencephalogram (EEG) frequencies while inhibiting others. It is a form of biofeedback associated with a particular aspect of the brain's electrical activity, such as frequency, location, amplitude, or duration of the EEG. A fundamental premise for NFB training is the existence of an orderly relationship between some aspects of EEG activity and a related clinical condition or behavioral state, and the effectiveness of NFB training in modifying or controlling that EEG pattern (155). Thanks to algorithms such as the Fast Fourier Transform, Compact Spectral Array (CSA), and advancements in computer memory and processing speed, real-time analysis of various EEG frequency bands is now possible (156). This enables nearly immediate EEG feedback as the subject's signal is generated, thus retaining its informative and reinforcing capacity.

As for other types of signals, they stand out hemoencephalographic (HEG) (157), and with, Functional Magnetic Resonance Imaging (fMRI) (158).

A Brain-Computer Interface (BCI) is a system that establishes a direct connection between brain activity and a computer. Its primary function is to enable a person to control or communicate actions with a machine or digital device through brain signals. In the context of EEG-based neurofeedback, a BCI is used to capture and process the electrical signals generated by the brain, as recorded in the electroencephalogram (EEG). These EEG signals are analyzed in real-time and presented visually to the user. The relationship between a BCI and neurofeedback lies in the fact that the BCI provides real-time feedback of the individual's brain activity, allowing them to become aware of their mental states and learn to self-regulate them. Users can apply specific strategies and techniques to modify their patterns of brain activity and achieve therapeutic goals, such as reducing stress, improving concentration, or controlling certain neurological conditions. In summary, a BCI serves as the tool that facilitates the EEG-based neurofeedback process, enabling individuals to consciously and therapeutically influence their brain activity (154).

1.2.3.2. NEUROPHYSIOLOGICAL EFFECTS OF NEUROFEEDBACK

The cortical areas of the brain generate different electrical rhythms, which are observed as signals in electronic recordings or EEG. These signals are divided into different categories (159):

- Delta (1-4 Hz) (160).
- Theta (4-8 Hz) (161).
- Alpha (8-12 Hz) (162).
- Beta (13-30 Hz): (162).
  - -Beta I (15-20 Hz).
  - -Beta II (20-30 Hz).
- Gamma (>35 Hz): (163).

As per Sherlin et al. (164), a comprehensive understanding of the functioning of these cerebral oscillations is crucial for establishing a sound justification for the results achieved through neurofeedback. This underscores the importance of possessing expertise in neurophysiology and neuroscience, as emphasized by Raymond et al.(165), when implementing this approach in a multidisciplinary context.

Neurofeedback (NFB) operates on the principle of operant conditioning, where the individual learns to increase or decrease the power of selected EEG frequency bands The changes in brain activity are typically mediated with visual or auditory feedback.

The neurophysiological mechanisms behind NFB are complex and multifaceted. One key mechanism is Hebbian plasticity, a process where simultaneous activation of neurons leads to pronounced increases in synaptic strength among those neurons. Another mechanism is homeostatic plasticity, which maintains the stability of neural circuits by adjusting synaptic strength inversely to recent changes in neuronal excitability (166).

NFB has been employed as a method to modulate distinct EEG frequencies for multifaceted objectives. Specifically, within the alpha band (8-12 Hz), NFB interventions have been utilized to facilitate changes associated with relaxation, attentional enhancement, and stress reduction. In the theta band (4-7 Hz), NFB protocols have been applied to induce states conducive to creative ideation, meditative states, and deep relaxation. In the beta band (13-30 Hz), NFB techniques have been utilized to target improvements in attentional focus, concentration, and cognitive performance. Nonetheless, it is crucial to acknowledge that the impact of NFB interventions may extend beyond the explicitly trained EEG frequencies, exerting broader effects on neural function and connectivity

1.2.3.3. NEUROFEEDBACK IN POST-STROKE REHABILITATION

The findings from one systematic review (167) suggest that stroke patients, similar to healthy individuals, can learn to control brain activity through neurofeedback, potentially leading to improvements in stroke symptoms. This observation is supported by studies focused on modulating brain activity and connectivity in stroke using techniques like functional Near-Infrared Spectroscopy (fNIRS), Magnetoencephalography (MEG), and Electroencephalography (EEG). The EEG (electroencephalogram) provides a high temporal resolution, measuring changes in brain activity in real-time with a temporal resolution on the order of milliseconds (ms). This high temporal resolution allows it to capture rapid electrical fluctuations in the brain, making it essential for studying processes like perception, cognition, and sensory responses. Notably, EEG neurofeedback has shown promise in cognitive and motor rehabilitation for stroke; however, its effects vary among participants. The authors propose that real-time functional Magnetic Resonance Imaging (rt-fMRI) may offer more precise feedback compared to EEG/MEG and fNIRS due to its superior spatial resolution, potentially making it easier for participants to learn how to control their brain activity or connectivity.

- <u>COGNITIVE REHABILITATION</u>

The available literature on NFB as a form of Cognitive Rehabilitation Therapy (CRT) within the stroke population is limited and doesn't strongly support NFB as an evidence-based treatment due to the limited quality and strength of the interventions conducted. Despite these limitations, some modest cognitive improvements were observed among participants. It's worth noting that NFB offers highly individualized treatment and integrates pre-post Quantitative EEG (QEEG) data, allowing for in-depth brainwave analysis specific to the user (168). Timing of NFB treatment initiation in these studies is noteworthy as it included participants in the sub-acute and chronic recovery phases post-stroke. However, it's challenging to attribute improvements solely to NFB due to the heterogeneous nature of stroke, prior therapies, and motivational factors, which may have played significant roles (169,170).

A 2017 review points out several limitations in the studies, including the lack of standardized recovery phase definitions, the heterogeneous nature of stroke, the absence of long-term follow-up data, and potential bias and confounding factors. The diverse NFB protocols and cognitive domains targeted across studies make cross-study comparisons challenging. In conclusion, while some cognitive improvements were observed with NFB in stroke patients, the review emphasizes the need for more rigorous and standardized research in this area to draw more definitive conclusions about the effectiveness of NFB as a CRT for stroke patients (169).

- <u>MOTOR REHABILITATION</u>

Recent research in neuroplasticity and technological advancements have unveiled new possibilities in post-stroke motor rehabilitation (171). Novel rehabilitation tools, such as Brain-Computer Interfaces (BCI) and Neurofeedback (NFB), are among the techniques used to stimulate neural plasticity and enhance functionality (172). This technology has demonstrated potential in post-stroke recovery when integrated with conventional therapies, mitigating maladaptive plasticity, amplifying motor cortex activity, and enhancing clinical outcomes (173).

Several studies (174,175) have shown the clinical efficacy of BCI in post-stroke upper limb rehabilitation. Early research supported the feasibility and effectiveness of BCI combined with Functional Electrical Stimulation (FES) (176) and Motor Imagery (MI)-based BCI (174,177) in conjunction with physiotherapy and robotic orthoses for post-stroke motor recovery. Recent studies have reaffirmed these findings, emphasizing a progression from self-guided movement (MI-based BCI) to assisted movement (174) (BCI-FES and BCI with orthoses) in enhancing upper limb motor function (178). MI, especially when combined with BCI, has shown promise for individuals with mild-to-moderate stroke.

The adaptation of NFB therapy for post-stroke recovery, considering the individual clinical situation of the patient, is very important, which could facilitate a more effective and personalized upper limb motor rehabilitation.

The integration of this technique qith new technological advances such as virtual reality, enhances the attractiveness of training and expedites the training process (179). Additionally, other advantages of this combination encompass: (1) enhancing patient engagement, (2) providing a more profound immersive experience, (3) diminishing the training duration due to increased intuitiveness in control, and (4) enabling therapy to take place within a confined physical area.

The NeuRow VR-BCI system, used in this thesis, is one of the most interesting paradigms published using the integration of NFB and VR. It utilizes a head-mounted VR headset with a wide field of view and haptic feedback delivered through hand controllers. Patients are immersed in a virtual canoe scenario where they control the direction of the boat by imagining the movement of their arms, which is visually represented in the virtual environment. The system reduces cognitive load and increases immersion, aiming to help patients perform correct movements efficiently. During training, patients receive feedback and aim to perform as many accurate movements as possible within a specified timeframe, with haptic feedback provided to activate the sensorimotor system when imagining specific movements (Figure 13) (180).

Figure 1313. MI-based NFB Application in a Patient with Chronic Stroke. Self-generated.

1.2.2.4. ADVANTAGES AND LIMITATIONS OF THE CLINICAL APPLICATION OF NFB IN STROKE

-<u>Advantages:</u>

- Customized Treatment: NFB enables the tailoring of treatment on an individual basis. A specific protocol can be designed based on the post-stroke patient's unique needs and capabilities, facilitating a personalized approach to address cognitive and motor deficits (181).

- Promotion of Neuroplasticity: Stroke often results in brain damage, and NFB can aid in fostering neuroplasticity, the brain's capacity for reorganization and recovery. By training patients to regulate their brain activity, damaged neural connections can be strengthened, potentially improving overall brain function (182).

- Symptom Reduction: In certain cases, NFB has demonstrated effectiveness in reducing symptoms associated with stroke, such as muscle weakness, coordination problems, fatigue, depression, and anxiety (173).

- Minimal Side Effects: Compared to some pharmacological treatments, NFB tends to have minimal side effects, rendering it a less invasive option for stroke survivors (173).

-<u>Limitations:</u>

- Patient Heterogeneity: Each stroke survivor is unique, with varying degrees of brain damage and affected areas. This poses a challenge in designing effective NFB protocols, as there is no one-size-fits-all approach (169).

- Cost and Accessibility: NFB can be costly and may not be readily available in all locations or for all patients, limiting its accessibility, especially for individuals with limited financial resources (183).

- Limited Evidence: Despite promising research on the use of NFB in stroke rehabilitation, the evidence in terms of controlled clinical trials and long-term studies remains limited compared to other rehabilitation approaches (184).

- Requires Commitment and Time: NFB entails regular training sessions that necessitate patient cooperation and commitment. Some patients may find it challenging or exhausting, particularly if they have cognitive or attention impairments (173).

In summary, NFB may offer potential benefits in the rehabilitation of stroke survivors by promoting brain plasticity and addressing physical and cognitive symptoms, but it also presents challenges related to customization, accessibility, and scientific evidence. Its clinical application should be carefully evaluated on a case-by-case basis, considering the individual needs and circumstances of the patient. Furthermore, it should be integrated as part of a comprehensive rehabilitation approach that includes other methods and therapies as needed.

The scientific evidence presented thus far has laid the foundation for this thesis, which offers a comprehensive review of the most efficient method for applying rTMS in the rehabilitation of upper limb sequelae during the late subacute and chronic phases following a stroke. Furthermore, we intend to amplify its efficacy by integrating a motor imagery and neurofeedback-based treatment within a BCI system. Our approach will encompass not only clinical but also neurophysiological effects, and ultimately, we will explore the relationship between the clinical and neurophysiological changes produced by the proposed intervention.

2. OBJECTIVES AND HYPOTHESIS

2.1. OBJECTIVES

The main objective of this doctoral thesis is to investigate the effects of a non-invasive multimodal brain neuromodulation approach in addressing UL chronic motor sequelae in patients who have suffered a stroke. The aim is to establish the efficacy of combining existing neuromodulation protocols to potentiate the effects of conventional rehabilitation. This general objective is developed through four independent studies that correspond each one to a specific objective:

- **Specific Objective 1**

To review the current available evidence regarding the effects of rTMS in improving upper limb motor function in late subacute and chronic stroke patients. Additionally, as a secondary objective, to review and determine the optimal stimulation parameters for enhancing motor response.

- **Specific Objective 2**

To design a clinical trial to study the clinical impact combining MI-based NFB training, using the NeuRow system (NeuroRehabLab, Funchal, Portugal), following bilateral rTMS neuromodulation in comparison to bilateral rTMS alone for the rehabilitation of UL motor sequelae after suffering a stroke with more than 3 months of evolution.

- **Specific Objective 3**

To examine the clinical effects induced by combining MI-based NFB training with bilateral repetitive transcranial magnetic stimulation for UL motor function in late subacute and chronic stroke patients.

- **Specific Objective 4**

To analyze the changes on neurophysiological measures such as interhemispheric cerebral asymmetry measures and cortical excitability in response to the application of the designed protocol for post-stroke UL rehabilitation using a combination of non-invasive brain stimulation techniques in late subacute and chronic patients.

2.2. HYPOTHESIS

The general hypothesis underlying this thesis is that the application of two different sequences of combined non-invasive neuromodulation using bilateral rTMS and EEG MI-based neurofeedback-VR on subacute and chronic stroke patients will have a significant positive impact on UL rehabilitation with differential effects depending on the sequence of application. To confirm this general hypothesis, three specific hypotheses have been proposed, which have been developed in three independent studies:

- **Hypothesis I**

Repetitive transcranial magnetic stimulation (rTMS) is a secure and practicable method for augmenting motor response and improving functionality in patients with upper limb (UL) chronic motor deficits post-stroke. This hypothesis will be assessed through a systematic review of the available literature.

- **Hypothesis II**

It is feasible to develop a combination of MI-based neurofeedback (NFB) training protocol and bilateral repetitive transcranial magnetic stimulation (rTMS) to enhance their neurophysiological and clinical effects in patients with chronic upper limb (UL) motor deficits following a stroke. This hypothesis will be evaluated through the design of a clinical trial protocol for the treatment of these deficits.

- **Hypothesis III**

A motor imagery (MI)-based neurofeedback (NFB) training protocol has the potential to augment the impact on motor performance, functional assessment, and somatosensory perception of the upper limbs produced by bilateral repetitive transcranial magnetic stimulation (rTMS) and conventional rehabilitation. This hypothesis will be assessed through the implementation of a clinical trial.

- **Hypothesis IV**

Basal Interhemispheric cerebral asymmetry and cerebral excitability are good neurophysiological markers determining the effectiveness or reflecting the efficacy of functional recovery after the application of a combination of MI-based neurofeedback (NFB) training protocol and bilateral repetitive transcranial magnetic stimulation (rTMS) to enhance their neurophysiological and clinical effects in patients with chronic upper limb (UL) motor deficits following a stroke.

3. MATERIALS, METHODS AND RESULTS

3.1. OBJECTIVE 1: TO REVIEW THE CURRENT AVAILABLE EVIDENCE REGARDING THE EFFECTS OF RTMS IN IMPROVING UPPER LIMB MOTOR FUNCTION IN LATE SUBACUTE AND CHRONIC STROKE PATIENTS.

3.1.1. IDENTIFICATION: SEARCH STRATEGY AND SOURCES

Searches were conducted across four electronic databases: Web of Science, PubMed, Scopus, and PEDro. Citation tracking for eligible articles was performed manually. The search was executed on July 13th, 2022. The search terms were based on three main concepts: rTMS, upper limb motor function, and stroke. Search strings varied slightly depending on the MeSH terms unique to each database. The chosen terms included: (rTMS OR 'repetitive transcranial magnetic stimulation' OR 'Theta Burst') AND (stroke OR 'cerebrovascular accident' OR CVA) AND 'motor cortex' AND (upper limb or hand) with no temporal restrictions.

3.1.2. ELIGIBILITY CRITERIA AND SELECTION

The eligibility criteria were defined as follows:

Inclusion criteria:

- Articles had to be peer-reviewed.
- The articles could be written in English, French, or Spanish.
- The study design had to be a clinical trial, including parallel and crossover trials.
- The intervention should involve rTMS applied to M1, of any type (unilateral/bilateral, high/low frequency, theta burst).
- Participants in the study had to be individuals who had experienced either an ischemic or hemorrhagic stroke and were in the subacute phase (3–6 months) or chronic phase (> 6 months) of recovery.
- The study had to provide outcomes related to UL motor function.

Exclusion criteria:

- Protocols for randomized trials, pilot studies, or observational studies were not considered.
- Studies involving other non-invasive (transcranial direct current stimulation) and invasive (epidural electrical stimulation) brain stimulation interventions were excluded.
- Studies focused on animal models of stroke or pediatric stroke were not included.
- Articles that did not provide measures of upper limb outcomes were excluded.

Following the removal of duplicates, the search results underwent a two-stage screening process based on the title and abstract, which was conducted by two authors. Proceedings, conference articles, book chapters, posters, and editorials were excluded before the full-text screening. Any disagreements were resolved through consensus, and if a consensus could not be reached, a third author had the deciding vote.

3.1.3. DATA EXTRACTION AND ANALYSIS

After applying the selection criteria, full-text articles underwent screening to extract information from each study. A data extraction table was developed using Microsoft Excel, encompassing various aspects of each study:

- Study characteristics, including the year of publication, study design, primary objective, and the presence of a control group.
- Sample size.
- Participant characteristics, which included age, gender, type of stroke (ischemic or hemorrhagic), lesion location (cortical or subcortical), and recovery phase (subacute or chronic).
- Details about the intervention, such as the total number of sessions and session frequency (sessions per week).
- rTMS parameters, including frequency (in Hz), intensity (percentage of the action/resting - motor thresholds), and the total number of pulses administered.
- Outcome measures related to UL motor function.
- The main findings of each study.

3.1.4. METHODOLOGICAL QUALITY ASSESSMENT

The PEDro scale, as described by Maher et al. (185) and Sherrington et al. (186), was employed in this study. This scale comprises 11 items designed to rapidly assess the internal validity and statistical adequacy of clinical trials, making their results more interpretable. Each item is scored as either 1 point (indicating that the criterion is met) or 0 points (indicating that the criterion is not met). Consequently, higher scores on the scale indicate greater methodological quality. The first item on the scale, which pertains to the source of participants and selection criteria, does not receive a numeric value. Thus, the scale yields scores ranging from 0 to 10 points.

Each article was independently evaluated by two assessors. To gauge the level of agreement between the two assessors, the kappa value (k) was calculated and interpreted as demonstrating high, moderate, or low levels of agreement when k was > 0.7, between 0.5 and 0.7, or less than 0.5, respectively.

3.1.5. RESULTS

3.1.5.1. SELECTION OF STUDIES

The PRISMA diagram (Figure 14) illustrates the information flow. Initially, we identified 1164 results from our searches, and after eliminating 559 duplicate records, we were left with 605 unique records. We screened these 605 records based on their titles and abstracts, which resulted in the removal of 504 records. This left us with 101 full-text articles that underwent further eligibility screening. Among these, 69 articles were excluded (see Figure 14). Eventually, we included a total of 32 articles that met our selection criteria.

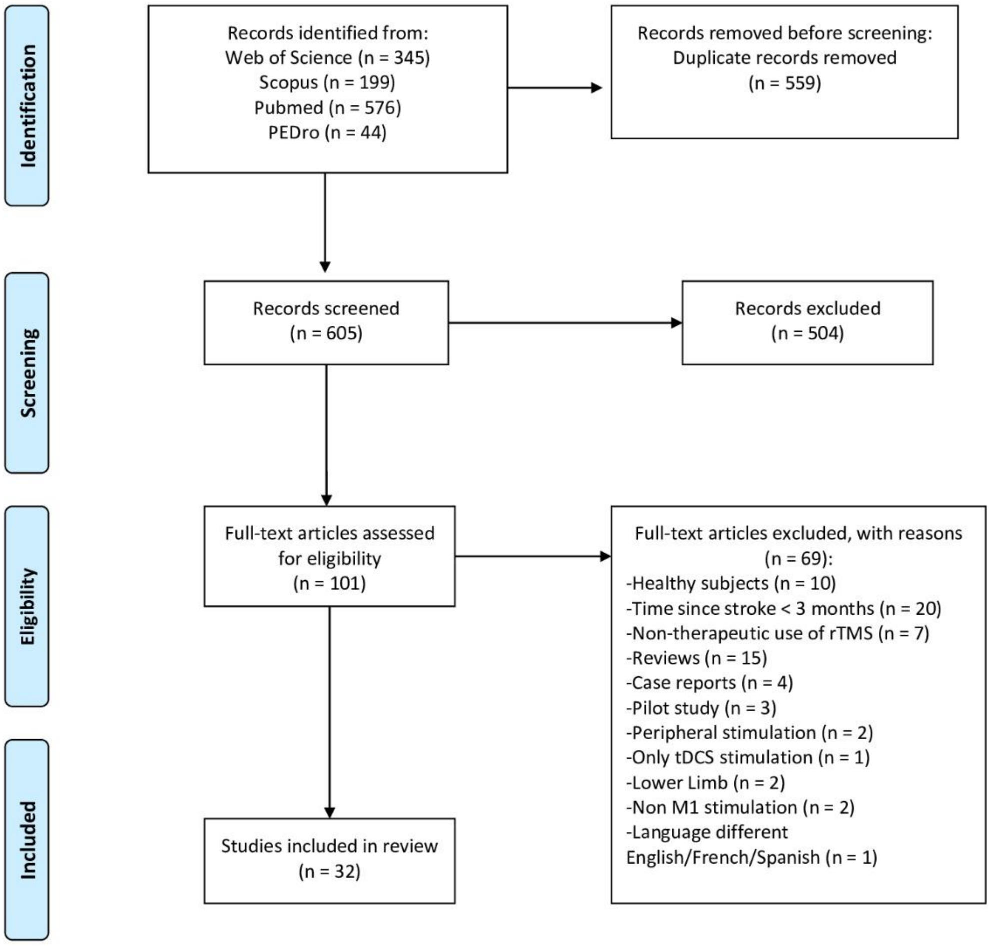

Figure 14.PRISMA flow diagram illustrating the selection process and flow of information.

3.1.5.2. CHARACTERISTICS OF INCLUDED STUDIES

Our review encompasses 32 studies, 24 of which employed a parallel group design, and 8 used a crossover design. In 30 studies, participants were randomly assigned to interventions, while 2 did not employ randomization. Twenty-nine studies featured a control group, while 3 did not (see Table 2).

Table 2. Characteristics of the included studies.

Study	Design	Objective	Sample size (Mean ± SD Age, years)	Gender (F/M)	Control Group	Lesion Location /Stroke type	Stroke Evolution Time
Du et al. (104)	Randomized, single-blinded, controlled trial.	To investigate the effect of neuromuscular electrical stimulation (NMES) combined with rTMS on poststroke hemiparetic upper limb rehabilitation.	240 (58 ± 36.2)	116/124	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
Zhang et al. (88)	Randomized, single-blinded, controlled trial.	To investigate the effects of priming iTBS followed by iTBS + robot-assisted training on poststroke hemiparetic upper limb recovery	42 (61 ± 7.7)	18/24	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
Yamada et al. (105)	Open-label, crossover, controlled study.	To compare the effects of the Program NEURO (rTMS + Occupational therapy) in patients with hemiparesis after stroke	37 (63 ± 8.1)	14/23	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
Kuzu et al. (187)	Randomized, double blinded, and controlled clinical trial.	To examine the effect of cTBS and low frequency rTMS on upper extremity spasticity and functional recovery in stroke patients.	20 (60.8 ± 8.6)	8/12	Yes	Cortical-Subcortical/Ischemic	Chronic
Chen et al. (188)	Randomized, single-blinded, controlled trial	To investigate the additive effect of iTBS on virtual reality-based cycling training (VCT) for upper limb function after stroke	23 (52 ± 9.8)	5/18	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Subacute
Haghighi et al. (189)	Randomized, parallel, double-blinded controlled trial	To determinate the effects of a rehabilitation program (RP) in conjunction with rTMS on paretic upper limb function after stroke	20 (52 ± 11.4)	9/11	Yes	Subcortical/ Ischemic-Hemorrhagic	Subacute
Pan et al. (190)	Randomized, single-blinded, controlled trial.	To investigate the effects of low frequency rTMS stimulation combined	42 (63 ± 5.2)	14/28	Yes	Cortical-subcortical/ Ischemic	Subacute

		with motor imagery (MI) on upper limb function after stroke.					
Harvey et al. (191)	Randomized, multicenter, blinded, sham-controlled trial.	To determine the effects of low-frequency electric field navigated rTMS to non-injured M1 improve arm motor function in hemiplegic stroke patients when combined with motor training.	199 (58.7 ± 13.1)	69/130	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Subacute/ Chronic
Johnson et al. (106)	Randomized, open label, controlled trial study	To evaluate the efficacy of combined rTMS + BCI, compared to sham rTMS + BCI, on motor recovery after stroke.	3 (55 ± 13)	2/1	Yes	Subcortical/Ischemic	Chronic
Askin et al. (107)	Single-blinded, randomized controlled study.	To assess the efficacy of inhibitory rTMS in healthy hemisphere M1 on upper extremity motor recovery and functional outcomes in chronic ischemic stroke patients.	40 (58 ± 11.7)	11/29	Yes	Cortical-subcortical/ Ischemic	Chronic
Cho et al. (192)	Randomized, open label, parallel study.	To investigate the effect of simultaneous bilateral stimulation using rTMS and tDCS over the bilateral M1 for the recovery of motor function.	30 (59 ± 10.6)	13/17	Yes	Cortical-Subcortical/Ischemic-Hemorrhagic	Subacute
Bashir et al. (108)	Open-label, controlled study.	To compare the effects of navigated rTMS between stroke patients and control subjects.	16 (62 ± 11.1)	8/8	No	Cortical-Subcortical/Ischemic-Hemorrhagic	Chronic
Cassidy et al. (109)	Randomized, crossover, single-blinded design	To evaluate changes in cortical excitability and paretic hand function in chronic stroke following three types of primed low frequency rTMS treatments on healthy hemisphere M1.	11 (64 ± 9.4)	3/8	Yes	Cortical-Subcortical/Ischemic-Hemorrhagic	Chronic
Demirtas-Tatlidede et al. (110)	Open-label, single-arm study.	To investigate the safety of contralesional M1 rTMS in a selected group of severe chronic stroke patients.	10 (59.5 ± 11)	6/4	No	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic

Wang et al. (193)	Randomized, double-blinded, controlled, parallel study.	To test the long-term efficacy of different sequences of 1 Hz rTMS on the healthy hemisphere M1 with contralateral iTBS to identify the strategy producing the most intense and long lasting electrophysiological / motor improvements in the paretic hand.	48 (62.6 ±12.5)	10/38	Yes	Cortical-subcortical/ Ischemic	Subacute
Rose et al. (111)	Randomized, double-blinded, controlled study.	To assess the effects of low frequency rTMS in healthy hemisphere M1 as an adjuvant to functional task practice, to improve upper extremity function.	19 (64.7 ± 8.0)	6/13	Yes	Cortical-subcortical/ Ischemic	Chronic
Kwon et al. (112)	Randomized, single-blinded, crossover study.	To determinate the most effective method for combining rTMS and motor training in stroke patients.	14 (53 ± 12.4)	3/11	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
Barros Galvao et al. (113)	Randomized, double-blinded, controlled trial.	To assess the efficacy of inhibitory rTMS for decreasing upper-limb muscle tone after chronic stroke.	20 (61 ± 9.4)	7/13	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
Carey et al. (114)	Randomized, single-blinded, controlled trial.	To analyse the characteristics of responder vs. non responders receiving rTMS to improve hand function after stroke.	12 (69 ± 10.1)	4/8	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
Etoh et al. (115)	Randomized double-blinded crossover study	To investigate whether multiple sessions of 1-Hz rTMS on healthy hemisphere M1 facilitates the effect of repetitive facilitation exercises on hemiplegic upper-limb function in chronic stroke patients.	18 (59.7 ± 11)	4/14	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
Tretriluxana et al. (116)	Randomized, single-blinded,	To investigate the effect of inhibitory low frequency rTMS applied to the non-lesioned hemisphere on kinematics and	9 (59 ± 6.8)	4/5	Yes	Cortical-subcortical/	Chronic

	controlled, crossover study	coordination of paretic arm reach-to-grasp action.				Ischemic-Hemorrhagic	
Sung et al. (117)	Randomized, single-blinded, controlled study.	To investigate the potential for a consecutive suppressive-facilitatory rTMS protocol to improve motor outcomes after chronic stroke.	54 (63 ± 12.1)	13/41	Yes	Cortical Subcortical / Ischemic-Hemorrhagic	Chronic
Avenanti et al. (118)	Randomized, double-blinded, controlled, study.	To investigate the long-term behavioural and neurophysiologic effects of rTMS on healthy hemisphere M1 combined with physical therapy intervention in chronic stroke patients with mild motor disabilities.	30 (63 ± 9.53)	14/16	Yes	Cortical-Subcortical / Ischemic-Hemorrhagic	Chronic
Talelli et al. (119)	Semi-randomized, single-blinded, controlled trial.	To explore whether long-lasting clinically important gains can be achieved by adding TBS to a rehabilitation program for the hand.	41 (56 ± 13.7)	20/21	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
Ackerley et al. (120)	Double-blinded, controlled, crossover study.	To determine the effects of M1 TBS and standardized training on upper-limb function of patients with chronic stroke.	10 (60 ± 11)	7/3	Yes	Subcortical/ Ischemic-Hemorrhagic	Chronic
Takeuchi et al. (121)	Randomized, double-blinded, study.	To investigate whether bilateral rTMS stimulating injured M1 and inhibiting healthy M1 may improve the paretic hand in patients after stroke.	30 (59 ± 12.4)	8/22	No	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
Takeuchi et al. (122)	Randomized, double-blinded, controlled study.	To investigate whether 1 Hz TMS on healthy hemisphere M1 improve the motor learning of the affected hand in patients after stroke.	20 (62.3 ± 8.4)	4/16	Yes	Subcortical/Ischemic	Chronic
Malcolm et al. (123)	Randomized, double-blinded, controlled study.	To test the potential adjuvant effect of rTMS on motor learning in a group of survivors undergoing constraint-induced therapy (CIT) for upper limb hemiparesis.	19 (67 ± 6.8)	8/11	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic

Fregni et al. (124)	Randomized, single-blinded, controlled study	To evaluate whether five sessions of low frequency rTMS on healthy hemisphere M1 produces improvements in upper limb motor function and whether this approach is safe and its effects durable	15 (56 ± 11.5)	4/11	Yes	Cortical-subcortical/ Ischemic	Chronic
Kim et al. (125)	Pseudorandomized, crossover, controlled study.	To investigate high-frequency rTMS-induced cortical excitability and the associated motor skill acquisition in chronic stroke patients.	15 (54 ± 4.5)	2/13	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
Takeuchi et al. (126)	Randomized, double-blinded, controlled study.	To study if 1 Hz rTMS on healthy hemisphere M1 improves motor performance of the affected hand in stroke patients by decreasing the transcallosal inhibition	20 (59.0 ± 9.6)	5/15	Yes	Subcortical/Ischemic	Chronic
Mansur et al. (127)	Double-blinded, controlled, crossover, study.	To investigate the use of low frequency rTMS on the unaffected hemisphere to decrease interhemispheric inhibition of the lesioned hemisphere and improve motor function in patients after stroke.	10 (53 ± 18.1)	7/3	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic

Note: subacute stage: 3-6 months post-stroke; chronic stage: > 6 months post-stroke. Acronyms: rTMS: Repetitive Transcranial Magnetic Stimulation; iTBS: Intermittent Theta Burst; cTBS: Continuous Theta Burst Stimulation; M1: Primary Motor Cortex.

3.1.5.3. PARTICIPANT CHARACTERISTICS

Our study involved a total of 1137 participants, with an average age of 59.5 ± 10.4 years, including 711 men. This sample reflects the sociodemographic characteristics typically observed in individuals who have experienced a stroke. Among the studies, 23 included participants with both ischemic and hemorrhagic strokes, while 9 included only those with ischemic strokes. Twenty-seven studies enrolled participants with cortical or subcortical lesions, and 5 studies included only individuals with subcortical lesions. Additionally, 26 studies included participants in the chronic stage of stroke, while 6 studies included those in the subacute phase (see table 2).

3.1.5.4. RTMS AND CONTROL INTERVENTIONS

- **rTMS Interventions**

Regarding the frequency of stimulation, 73% of the studies applied low-frequency rTMS to the contralateral M), while 16% used high-frequency rTMS on the ipsilateral M1. Only 20% of the studies employed iTBS to stimulate the ipsilateral M1 (see Table 3). The most common frequencies used were 1 Hz for low-frequency inhibitory rTMS and 10 Hz for high-frequency facilitatory protocols. The total number of pulses varied from 160 to 2400, with 15 studies using more than 1000 pulses. Regarding the stimulation threshold, three studies used 100% of the resting motor threshold, while the rest used thresholds ranging from 90% to 80%.

- **Control Interventions**

Among the studies, 21 utilized sham TMS as the control intervention. Five trials employed conventional rehabilitation, and one study used an audiotape featuring a relaxation program with soothing music. Two studies used rTMS as a control intervention when comparing it to rTMS combined with adjuvant therapy. In one study, unilateral application with rTMS was utilized, and two studies did not include a control group (see table 3).

3.1.5.5. OUTCOME MEASURES

All the studies focused on evaluating arm/hand motor function using various scales or instrumental assessments. Additionally, spasticity, cognition, and/or neurophysiological variables were assessed. The assessment metrics exhibited significant heterogeneity across studies. Assessments were conducted both before and after treatment.

Motor function assessments employed diverse metrics, which were categorized based on the constructs they evaluated. General upper limb motor function was assessed using the Fugl-Meyer Assessment-UL (FMA-UL) in 15 studies, the Wolf Motor Function Test (WMFT) in 9 studies, and the Motor Activity Log (MAL) in 5 studies. UL strength was evaluated in two studies using the Medical Research Council (MRC) scale. Gross manual dexterity was assessed using the Box and Blocks Test (BBT) in 7 studies. Fine manual dexterity was measured with the Nine Hole Peg Test (NHPT) in 12 studies, the Action Research Arm Test (ARAT) in 7 studies, the Purdue Pegboard Test (PPT) in 3 studies, and the Jebsen Taylor Hand Function Test (JHFT) in 3 studies.

Concerning spasticity, its presence and severity were assessed in 9 studies using the Modified Ashworth Scale (MAS). Instrumental measures included handgrip strength assessed by 5 studies using dynamometry and pinch strength assessed by 7 studies using a pinchmeter. Neurophysiological measures for detecting changes in cortical excitability were employed in 16 studies, utilizing various stimulation models and coils. Metrics included interhemispheric inhibition (IHI), intracortical inhibition (ICI), short interval intracortical inhibition (SICI), intracortical facilitation (ICF), ipsilateral silent period (ISP), cortical silence period (CSP), and MEPs.

3.1.5.6. EFFECTIVENESS OF RTMS ON UPPER LIMB MOTOR SEQUELAE

- **Comparison 1: Active rTMS vs. Sham rTMS**

Active rTMS groups showed significant improvements in FMA-UE, ARAT, STEF, NHPT, WMFT, MAL, BBT, JHFT, movement accuracy, movement time, movement acceleration, pinch force, and handgrip. Only 5 studies did not find significant differences between groups (111,116,119,191).

- **Comparison 2: rTMS vs. Conventional Rehabilitation**

rTMS groups demonstrated significant improvements in FMA-UL, FMA-TT, WMFT, MAS, MAL, SIS, BBT, grip and pinch strength, Modified Barthel Index (MBI), Functional Independence Measure (FIM), FAS, MMSE, and reaction time. Only one study did not find significant differences compared to the control intervention (112). Regarding the other control groups, those using rTMS without any additional adjuvant therapy or audiotape relaxation programs with soothing music showed significant improvement in the experimental group based on FMA-UL, WMFT, BBT, and MBI.

- **Comparison 3: High-Frequency rTMS vs. Low-Frequency rTMS**

Significant improvements favored contralateral low-frequency stimulation over ipsilateral high-frequency application for FMA-UE, grip strength, MAS, NHPT, BBT, WMFT, and SIS.

- **Comparison 4: Unilateral rTMS vs. Bilateral rTMS**

Experimental groups exhibited significant improvements in favor of bilateral protocols not only in acceleration and pinch force (121) but also in MRC, FMA-UL, WMFT, and reaction times (117).

- **Comparison 5: Active TBS vs. Sham TBS**

Stimulation with active TBS was significantly more effective than sham TBS according to FMA-UL, MAS, MAL, ARAT, and SIS, except in one study (119).

Table 3. Characteristics of the included studies according to dosage, transcranial magnetic stimulation parameters, outcome measures and main findings.

Study	Stimulation parameters		Outcome Measures	Main Finding
	Number of sessions	Frequency, Intensity and Pattern		
Du et al. (104)	20 (5 times /week x 4 weeks)	**Group A:** Control group, only routine treatment. **Group B**: NMES based on rehabilitation treatment. **Group C**: rTMS based on rehabilitation treatment (cM1, 1 Hz, 20 min, 1200 pulses; 90% RMT) **Group D**: NMES + rTMS (cM1, 1 Hz, 20 min, 1200 pulses; 90% RMT) based on routine treatment.	-Modified Barthel Index (MBI). -FMA-UE and MAS. - MEPs and Central motor conduction time (CMCT)	Group D showed statistically significant improvements ($p < 0.05$) in FMA, MAS, MEPs and CMCT.
Zhang et al. (88)	10 (5 times /week x 2 weeks)	**Group A:** Priming iTBS (iM1 cTBS at 70% RMT), followed by iTBS at 70% RMT + robot-assisted training. **Group B:** Nonpriming iTBS (iM1 cTBS at 20% RMT) followed by iTBS at 70% RMT + robot-assisted training. **Group C**: Sham stimulation (iM1 cTBS at 20% RMT) followed by iTBS at 20% RMT immediately before robot-assisted training.	-FMA-UE, ARAT. -The mean velocity of movement. -EEG.	Group A presented greater improvement than Group B and C in FMA-UE, ARAT, and mean velocity of movement. Only group A showed statistically significant differences in FMA-UE ($p < 0.05$).

Yamada et al. (105)	10 (5 times /week x 2 weeks)	**Group A:** Neuro Program (cM1 1 Hz rTMS (40 min, 2400 pulses; 90% RMT) followed by Occupational Therapy) **Group B:** Occupational Therapy	-FMA-UE, WMFT.	Group A showed statistically significant improvements (p<0.05) in FMA and WMFT.
Kuzu et al. (187)	10 (5 times /week x 2 weeks)	**Group A:** Active cM1 1Hz rTMS (20 min, 1200 pulses; 90% RMT) followed by Physical Therapy. **Group B:** Active cM1 cTBS (600 pulses; 80% AMT) followed by Physical Therapy. **Group C:** Sham cM1 cTBS (600 pulses; 80% AMT) followed by Physical Therapy	-FMA-UE and MAS. -FIM, MAL and Brunnstrom stage.	Group A and B showed significant improvements in FMA-UE, FIM and MAL (p<0.05), and non-significantly in MAS (p>0.05). Group C did not present significant improvements in any variable.
Chen et al. (188)	15 (5 times/week x 3 weeks)	**Group A:** Active iM1 iTBS (80% AMT) followed by 60 min of virtual reality-based cycling training (VCT) **Group B:** Sham iM1 iTBS (80% AMT) followed by 60 min of virtual reality-based cycling training (VCT)	-FMA-UE, MAS-UE, ARAT, NHPT, BBT, MAL and SIS.	Group A presented greater improvement than Group B in MAS-UE, MAL, and SIS (p < 0.05).
Haghighi et al. (189)	10 (3 times/week)	**Group A:** Active iM1 20 Hz rTMS (2000 pulses; 90% RMT) followed by a Rehabilitation Program (RP) **Group B:** Rehabilitation Program	-FMA, BBT. -Grip strength and pinch strength.	All outcome measure showed significant improvements (p < 0.05) regarding group A.

Pan et al. (190)	10 (5 times /week x 2 weeks)	**Group A:** Active cM1 1 Hz rTMS (1500 pulses; 90% RMT) followed by an Audio-Based Motor Imagery (30 min) **Group B:** Active cM1 1 Hz rTMS (1500 pulses; 90% RMT) followed by an Audiotaped-Led Relaxation (30 min)	-FMA-UE, WMFT, BBT and MBI.	They found greater change of FMA-UE, WMFT, BBT and MBI scores in group A ($p < 0.05$).
Harvey et al. (191)	18 (3 times/week x 6 weeks)	**Group A:** Active cM1 1 Hz rTMS (> 15minutes) followed by 60 min of task-oriented rehabilitation therapy. **Group B:** Sham cM1 1 Hz rTMS (> 15minutes) followed by 60 min of task-oriented rehabilitation therapy.	-FMA-UE, ARAT and WMFT.	All outcome measures showed significant improvement in both groups ($p<0.05$). Significant differences between groups were not found.
Johnson et al. (106)	18 (3 times / week x 6 weeks)	**Group A:** Active cM1 1Hz rTMS (10 min; 90% RMT) followed by BCI training (First 3 weeks) + BCI training (Second 3 weeks) **Group B:** Sham cM1 1Hz rTMS (10 min; 90% RMT) followed by BCI training (First 3 weeks) + BCI training (Second 3 weeks)	-BBT, FTT. -IHI, MEP -PVC	Increased ipsilesional motor activity and improvements in BBT and FTT for group A ($p < 0.05$).
Askin et al. (107)	10 (5 times /week x 2 weeks)	**Group A:** Physical therapy **Group B:** Active cM1 1 Hz rTMS (1200 pulses, 90% RMT) followed by Physical therapy	-MAS, Brunnstrom Stages, FMA, BBT. -FIM, FAS. -MMSE.	Regarding group B, there were statistically significant improvements in all clinical outcome measures except for the Brunnstrom Stage. MMSE scores were significantly increased in group B.

Cho et al. (192)	10 (5 times /week x 2 weeks)	**Group A:** Active iM1 10 Hz rTMS (20 min, 1000 pulses, 90% RMT) with the simultaneous application of cathodal tDCS (2mA) over cM1. **Group B:** Active iM1 10 Hz rTMS (20 min, 1000 pulses, 90% RMT)	-FMA-UE -FMA-LE -FMA-T	Significant differences were found in FMA-UE and FMA-T in the dual mode stimulation group ($p < 0.05$)
Bashir et al. (108)	1	**Patient Group:** Active cM1 1 Hz rTMS (1200pulses, 90% RMT) **Healthy Group:** Active right M1 1 Hz rTMS (1200pulses, 90% RMT)	-FTT, NHPT, Strength Index, Reaction time. -RMT, MEP, ICI, IF, CSP.	All participants improved FTT, strength index and increased excitability, but group A was more significative ($p < 0.05$)
Cassidy et al. (109)	1	**Group A:** Active cM1 6 Hz rTMS priming (10 min, 600 pulses, 90%RMT) + active cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT). **Group B:** Active cM1 1 Hz rTMS priming (10 min, 600pulses, 90%RM) + active cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT). **Group C**: Sham cM1 6 Hz rTMS priming (10 min, 600 total pulses, 90%RMT) + active cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT).	- RMT, SICI, IF and CSP. - MEP. - BBT.	No significant differences were found in outcome measure of group B and C. Regarding group A, there are significant improvements in: -Ipsilesional CSP ($p < 0.05$) -SICI from baseline ($p < 0.05$) -BBT ($p < 0.05$)

Demirta-tatlidede et al. (110)	10 (5 times /week x 2 weeks)	Active cM1 1 Hz rTMS (1600 pulses, 100% RMT)	- FMA, hand grip strength, MAS and WMFT. - MEP, SICI, ICF and TCI.	No significant differences were found in MAS, WMFT, MEP, SICI, ICF and TCI duration. Significant differences were found in FMA and hand grip strength ($p < 0.05$). Both improve compared to the previous evaluation. Changes persisted at follow up 1 month
Rose et al. (111)	16 (4 times /week x 4 weeks)	**Group A:** Active cM1 1 Hz rTMS (1200 pulses, 100% RMT) followed by 1 hour of functional task practice activities. **Group B:** Sham cM1 1 Hz rTMS (1200 pulses, 100% RMT) followed by 1 hour of functional task practice activities.	-WMFT, FMA, MAS, MAL, ARAT, LLFDI and ROM. -Grip force, Lateral Pinch force, Palmar Pinch force, and 3-Jaw Chuck Force. -Light Touch Sensation. -RMT, SICI, $V50$. -Movement time, trunk displacement and maximum resultant velocity.	No significant differences were detected for any of the clinical measures between the real-rTMS and sham-rTMS group. Post hoc small but statistically significant improvements in upper extremity behavioural measures. No significant differences were detected for SICI, $V50$ or RMT ($p>0.05$).
Kwon et al. (112)	1	**Group A:** Active iM1 10 Hz rTMS (20 min, 1000 pulses, 90% RMT) and simultaneous	-Movement accuracy and movement time.	All participants improved all outcome measure, but there isn't

		finger motor task during each inter-train interval. **Group B:** Active iM1 10 Hz rTMS (10 min, 1000 pulses, 90% RMT) and after stimulation, finger motor task.	-Purdue Pegboard Test and NHPT.	significant difference between both groups.
Wang et al. (193)	20 (5 times /week x 4 weeks)	**Group A:** 10 sessions Active cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT) followed by 10 sessions Active iM1 iTBS (190 seconds, 600 pulses, 80% RMT) + Conventional physiotherapy. **Group B**: 10 sessions Active iM1 iTBS (190 seconds, 600 pulses, 80% RMT), followed by 10 sessions Active cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT) + Conventional physiotherapy. **Group C:** 10 sessions Sham cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT) followed by 10 sessions Sham iM1 iTBS (190 seconds, 600 pulses) + Conventional physiotherapy	- FMA, WMFT, and MRC. - MEP.	No significant differences were found in outcome measure of group B and C. Regarding group A, there are significant improvements in: - MRC ($P < 0.05$) - FMA ($P < 0.05$) - WMFT ($P < 0.05$) The effects lasted at least 3 months.
Barros Galvao et al. (113)	10 (3 times/week)	**Group A:** Active Cm1 1 Hz Rtms (1500 pulses; 90% RMT) followed by Physical therapy.	-MAS, FMA, FIM. -SSQOL. -ROM.	In the group A, 90% of the patients at postintervention and 55.5% at 1 month follow-up showed a decrease of < 1 in the MAS score, representing clinically important differences.

		Group B: Sham Cm1 1 Hz Rtms (1500 pulses; 90% RMT) followed by Physical therapy.		There was no difference in the number of participants who showed clinically relevant changes between groups, except MAS.
Carey et al. (114)	5 (5 times/week)	**Group A:** Active Cm1 6Hz (10min, 600pulses, 90% RMT) + Active Cm1 1Hz (10 min, 600 pulses, 90% RMT) followed by tracking training. **Group B:** Active Cm1 6Hz (10min, 600pulses, 90% RMT) + Active Cm1 1Hz (10 min, 600 pulses, 90% RMT).	-TEMPA -NIHSS -FMA-UE, MMSE.	Results showed significant differences only in TEMPA ($p < 0.05$)
Etoh et al. (115)	20 (5 times /week x 4 weeks)	**Group A:** Active cM1 1Hz rTMS (4 min, 240 pulses, 90% RMT) for 2 weeks + Sham 5cm posterior to cM1 1Hz rTMS (4 min) for 2 weeks + repetitive facilitation exercises for 40 min during active and sham rTMS sessions **Group B:** Sham 5cm posterior to cM1 1Hz rTMS (4 min) for 2 weeks + Active cM1 1Hz rTMS (4 min, 240 pulses, 90% RMT) for 2 weeks + repetitive facilitation exercises for 40 min during active and sham rTMS sessions	-FMA, ARAT, MAS and STEF.	Significant differences were found in ARAT, FMA and STEF scores when motor facilitation was performed during active but not sham rTMS. No significant changes were found in MAS.

Tretriluxana et al.(116)	2	**Group A:** Reach to grasp task followed by active cM1 1Hz (20 min, 1200 pulses, 90%RMT). **Group B:** Reach to grasp task followed by sham cM1 1Hz (20 min, 1200 pulses, 90%RMT).	-Peak transport velocity, peak aperture, time of peak transport velocity, time of peak aperture and total movement time. -MEP.	Significant reduction in the MEP amplitude between groups (P < 0.05). There were no changes in Reach to Grasp kinematics.
Sung et al. (117)	20 (5 times /week x 4 weeks)	**Group A:** Active cM1 1Hz followed by active iTBS over iM1. **Group B:** Sham cM1 1Hz followed by active iTBS over iM1. **Group C:** Active cM1 1Hz followed by sham iTBS over iM1. **Group D:** Sham cM1 1Hz followed by sham iTBS over iM1.	-FMA-UE, WMFT. -MRC -Reaction Time and FTT -MEP	Only group A showed greater muscle strength (MRC), FMA-UE, WMFT and Reaction times in comparison with the other 3 groups (p < 0.05).
Avenanti et al. (118)	10 (daily sessions)	**Group A:** Active cM1 1 Hz rTMS (25 min, 1500 pulses, 90% RMT) **Group B**: Sham cM1 1 Hz rTMS (25min, 1500 pulses) Each protocol divided into 2 groups: stimulation before PT and stimulation after PT	-Tip-pinch, and power-grip force. - ISP, RMT. - JHFT, NHPT and BBT.	Significant differences were found in the outcome measures (JTT, NHPT, BBT and key grip) on both rTMS groups with bigger and longer lasting effects in the rTMS-PT group. iSP and rMT changed significantly in both rTMS groups. Effects persisted for 90 days

Talelli et al. (119)	10 (5 times /week x 2 weeks)	**Group A:** Active iM1 iTBS at 80% AMT followed by physical therapy. **Group B:** Sham iM1 iTBS at 80% AMT followed by physical therapy. **Group C:** Active cM1 cTBS at 80% AMT followed by physical therapy. **Group D:** Sham iM1 cTBS at 80% AMT followed by physical therapy.	-NHPT, JTT. -Grip and pinch-grip dynamometry.	There were no differences between the active treatment and sham groups in any of the outcome measure.
Ackerley et al. (120)	1	**Group A:** Active iM1 iTBS at 90% AMT followed by motor training. **Group B:** Active cM1 cTBS at 90% AMT followed by motor training. **Group C:** Sham TBS (to either M1) followed by motor training.	-Movement accuracy and movement time. -ARAT. -MEP.	Training after real TBS (Group A/B) improved paretic-hand grip-lift kinetics. ARAT improved in Group A but not in Group B, this was correlated with reduced ipsilesional corticomotor excitability.
Takeuchi et al. (121)	10 (5 times /week x 2 weeks)	**Group A:** Active cM1 1 Hz rTMS (1000 pulses, 90% RMT) +motor training after stimulation. **Group B:** Active iM1 10 Hz rTMS (1000 pulses, 90% RMT) + motor training after stimulation. **Group C:** Active cM1 1 Hz rTMS (1000 pulses) + Active iM1 10 Hz rTMS (1000	-ICI, RMT and MEP. -Acceleration and Pinch Force.	No significant improvement was found in outcome measure of group A and B. Regarding group C, there are significantly better outcomes in: -Acceleration ($p < 0.05$) -Pinch force ($p < 0.05$) -MEP (contralesional $p < 0.05$,

		pulses, 90% RMT) + motor training after stimulation.		ipsilesional $p < 0.05$) - ICI ($p < 0.05$)
Takeuchi et al. (122)	1	**Group A:** Active cM1 1 Hz rTMS (25 min, 1500 pulses, 90% RMT) + Motor training **Group B:** Sham cM1 1 Hz rTMS (25 min, 1500 pulses) + Motor training	-ICI, RMT and MEP. -Acceleration and Pinch Force.	In group A significant improvements were found in Acceleration and Pinch force. Contralesional and ipsilesional MEP increased ($p < 0.05$). No significant differences were found in RMT.
Malcolm et al.(123)	10 (5 times /week x 2 weeks)	**Group A:** Active iM1 20 Hz rTMS (2000 pulses, 90% RMT) + Constraint-Induced Therapy (CIT) **Group B:** Sham iM1 20 Hz rTMS (2000 pulses, 90% RMT) + Constraint-Induced Therapy (CIT)	-WMFT, MAL and BBT. -RMT/AMT.	Group A presented significant improvements in WMFT, MAL and BBT ($p < 0.05$). There was no significant difference in motor threshold between the groups.
Fregni et al. (124)	5 (daily sessions)	**Group A:** Active cM1 1Hz rTMS (20 min, 1200 pulses, 100% RMT) **Group B:** Sham cM1 1Hz rTMS (20 min, 1200 pulses)	-CRT, SRT, JHFT and PPT. - MMSE, Stroop test and digit span forward and backward. -RMT.	No significant differences were found in cRT, sRT PPT, Mini-Mental State Examination, Stroop test and digit span forward and backward. Significant differences were found in JHFT and RMT changes in the

				affected hemisphere ($p < 0.05$). The effects lasted 2 weeks.
Kim et al. (125)	1	**Group A:** Active iM1 10 Hz rTMS (160 pulses, 80% RMT) + Finger Motor Task **Group B:** Sham iM1 10 Hz rTMS (160 pulses, 80% RMT) + Finger Motor Task	-Movement accuracy and movement time. -MEP.	Group A showed significant differences in all outcome measures ($p < 0.05$).
Takeuchi et al. (126)	1	**Group A:** Active cM1 1 Hz rTMS (25 min, 90% RMT) **Group B:** Sham cM1 1 Hz rTMS (25 min) Motor training was done in both groups until one day before stimulation	-TCI duration, MEP and RMT. -Acceleration and Pinch Force.	The improvement of motor function (Acceleration and Pinch Force) after rTMS was significantly associated with the reduction of TCI duration in active rTMS group ($p < 0.05$).
Mansur et al. (127)	1	**Group A:** Active cM1 1 Hz rTMS (600 pulses, 100% RMT) **Group B:** Sham cM1 1 Hz rTMS (600 pulses, 100% RMT) **Group C:** Active premotor cortex 1 Hz rTMS (600 pulses, 100% RMT)	-sRT, cRT. -Purdue Pegboard Test. -Finger Tapping.	There were significant improvements in sRT, cRT and Purdue Pegboard Test respect group A ($p < 0.05$). There were improvements in group C, but no significant.

Acronyms: ARAT: Action Research Arm Test; BBT: Box and Blocks Test; cTBS: Continuous Theta Burst Stimulation; cM1: Contralateral Primary Motor Cortex; CRT: Choice Reaction Time; CSP: Cortical Silent Period; FAS: Functional Ambulation Scale; FIM: Functional Independence Measurement; FMA: Fugl Meyer Assessment; ICF/IF: Intracortical Facilitation; ICI: Intracortical Inhibition; IHI: Interhemispheric inhibition; iTBS: Intermittent Theta Burst Stimulation; iM1: Ipsilateral Primary Motor Cortex; ISP: Ipsilateral Silent Period; JHFT: Jebsen-Taylor Hand Function Test; LLFDI: Late-Life Function and Disability Index; MAL: Motor Activity Log; MAS: Modified Ashworth Scale; MEP: Motor Evoked Potential; MMSE: Mini-Mental State Examination; MRC: Medical Research Council; NHPT: Nine Hole Peg Test; NMES: neuromuscular electrical stimulation; NS: number of sessions; PPT: Purdue Pegboard Test; PT: Physical Therapy; Ref: references of the selected articles; RMT: Resting Motor Threshold; ROM: Range of Motion; rTMS: Repetitive Transcranial Magnetic Stimulation; SICI: Short Intracortical inhibition; SRT: Simple Reaction Time; SSQOL: Stroke Specific Quality of Life Scale; STEF: Simple Test For Evaluating Hand Function; TCI: Transcallosal inhibition; TEMPA: Test Evaluant la Performance des Membres supérieurs des Personnes âgées; V50: The stimulus intensity at which MEP amplitude is 50% of the MEPMAX and WMFT: Wolf Motor Function Test.

3.1.5.7. METHODOLOGICAL QUALITY

The included studies had an average PEDro score of 7.09±1.97 (F.S.C) and 7.22±1.80 (Y.G.Z) points. Figure 15 depicts the distribution of total scores for each article, as assessed by both reviewers. There was moderate agreement between the raters, with a kappa value (k = 0.61) indicating good overall methodological quality in the included studies. Higher PEDro scores were associated with the inclusion of a control group and the use of double-blind and/or randomized designs.

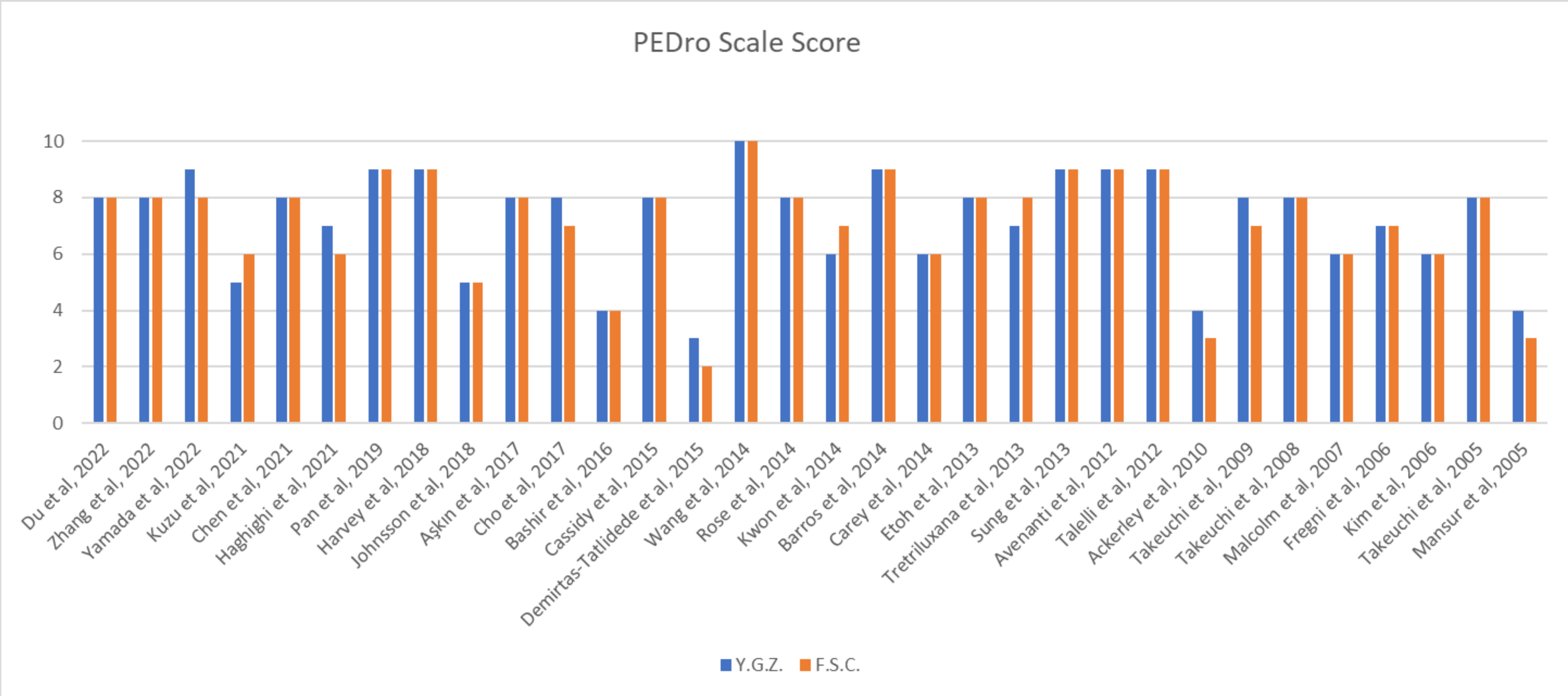

Figure 1514. Total PEDro score of the included studies assigned by each reviewer.

3.1.6. DISCUSSION

This systematic review analyzed the available clinical trial evidence to assess the effectiveness of rTMS on UL motor function in individuals in the subacute and chronic stages following a stroke. The results indicate that there is supporting evidence for its application in clinical practice. Across various variables examined in our review, regardless of the timing of treatment initiation, rTMS appears to have a positive impact on UL functionality, as assessed through functional tests. This aligns with existing literature that has reported favorable effects of rTMS during the acute phase of stroke recovery (194).

Recent systematic reviews with meta-analyses (194) have confirmed the effectiveness of rTMS in improving general UL motor function as measured by the FMA-UE. However, its influence on other aspects of motor function, such as strength, dexterity, or muscle tone, has been previously underexplored. In our review, we compile evidence suggesting that rTMS may also be effective in enhancing these aspects, which contribute to daily living activities. Furthermore, we aggregate evidence on the neurophysiological factors associated with these functional improvements by examining measures of cortical excitability.

Our review encompasses 32 studies that met our inclusion criteria and were published between 2005 and 2022. Most of these studies (n = 19) exhibited good methodological quality, as reflected by a PEDro score of ≥7, ensuring the validity of the conclusions drawn. However, there was substantial heterogeneity among the studies regarding sample size, location of brain lesions, and stroke types. Some studies had small sample sizes (n = 3), and most included fewer than 50 patients, with only one study involving 240 individuals. Additionally, there was consistent underreporting of sample size calculations, which could introduce statistical biases. While the location and size of brain lesions might influence the effectiveness of stimulation (195), most studies did not differentiate between patients with varying etiologies or lesion locations. Although nine studies exclusively included individuals with ischemic stroke, their results did not significantly differ from those including hemorrhagic stroke.

This review identified a higher number of low-frequency rTMS protocols applied to the contralesional hemisphere compared to high-frequency stimulation on the affected hemisphere (21 vs. 5 studies). Nevertheless, there is ongoing debate about the relative efficacy of these interventions and whether one is superior to the other in terms of clinical effects on UL motor function. Our review aligns with previous meta-analyses suggesting stronger effects of low-frequency rTMS compared to high-frequency stimulation in chronic stroke (194), although these findings are not conclusive. Furthermore, emerging evidence suggests that low-frequency stimulation on the unaffected hemisphere might concurrently disrupt ipsilateral motor commands, potentially impairing non-paretic UL function and bimanual coordination (196). While these findings are preliminary and need confirmation in larger clinical trials, researchers and clinicians should be attentive to the potential adverse effects of such rTMS approaches. More high-quality research is required to validate these results and provide evidence-based recommendations that consider the merits and drawbacks of unilateral rTMS interventions.

There is presently conflicting evidence regarding the superiority of bilateral protocols that combine low-frequency and high-frequency rTMS interventions compared to their isolated use. Our review found that bilateral stimulation yielded superior results compared to unilateral rTMS, but recent meta-analyses did not support this (194). Based on the studies in our review, bilateral rTMS showed greater effectiveness in improving acceleration, pinch force, MRC, FMA-UE, WMFT, and reaction times (117,121). Consequently, there is emerging evidence that bilateral protocols may also be effective in addressing certain UL motor impairments in the chronic stage following a stroke. Nevertheless, due to the limited number of trials investigating bilateral application, further research is warranted to explore its effects compared to unilateral protocols.

While safety guidelines are in place (197), there is no consensus on the most effective dosing parameters (intensity, number of pulses, or sessions) for stroke rehabilitation using rTMS. This contrasts with rTMS applications for depression and chronic pain, which have evidence-based recommendations regarding the stimulation site and frequency. Nevertheless, the safety of rTMS interventions appears to be well-established, as none of the included studies (unilateral or bilateral) reported severe adverse effects such as seizures. It should be noted that safety assessment was not the primary focus of these studies.

Regarding the number of sessions, prior studies suggest that five rTMS sessions may yield more favorable effects on UL function compared to a single session or more than 10 sessions (198). Our analysis revealed that the most common protocol consisted of 10 sessions (as seen in 13 studies) distributed over two weeks. However, there is evidence indicating beneficial effects on functional and physiological measures even with a single session (108,112,114,116,120,122,125,127). The low number of sessions was not associated with safety concerns. Nevertheless, the actual duration of the effects of rTMS protocols in stroke remains uncertain, as long-term follow-up (beyond three months) and the impact of repeated protocols have not been thoroughly evaluated.

Most stroke patients receive physical therapy as a fundamental component of their rehabilitation. Thirteen studies included muscle activation or physical therapy alongside rTMS treatment, and the results indicated that better outcomes were observed when physical activation was conducted concurrently (115) or following rTMS (88,105,107,111,113,118,121,122,187,191,193), with limited effects when performed prior to neuromodulation (118). Nevertheless, one study (88) utilized robot-assisted rehabilitation with and without iTBS priming, demonstrating the greater efficacy of the primed protocol, and two studies successfully tested the effect of 6 Hz priming before 1 Hz inhibition (109,114). These priming approaches may lead to better outcomes by combining various complementary neurophysiological mechanisms. This has prompted the exploration of different combinations of non-invasive brain stimulation techniques (199) or the pairing of cortical neuromodulation with subsequent motor learning (200). In the case of physical activity priming, fatigue could be a factor inhibiting cortical excitability (201), potentially impairing subsequent rTMS activation and resulting in poorer clinical outcomes compared to rTMS priming.

The lack of consistency in outcome measures for UL motor recovery makes it challenging to make comprehensive comparisons across different rTMS protocols. In addition to its effects on general UL motor function assessed by the FMA, our review identified positive effects on other specific domains contributing to overall function. Nine studies used the MAS to assess spasticity, with only five showing a positive effect (104,107,113,187,188).

Furthermore, our review revealed that rTMS not only improves functional measures related to the affected UL but also leads to generalized improvements(107,113,118,124,193), such as handgrip strength (110) and improvements in acceleration and pinch force (121,122). Additionally, some studies have suggested a potential link between cortical neurophysiological variables (e.g., resting motor threshold reduction, changes in MEP amplitude) and improved function in the affected UL after stroke (202,203). However, neurophysiological evaluations and measures of cortical excitability were not consistently related to clinical effects in most included studies, and vice versa. Only three studies demonstrated significant changes in neurophysiological metrics alongside substantial clinical and functional improvements (109,118,121). While cortical excitability measures have been correlated with motor performance following stroke (204), it appears that clinical improvement is not always associated with measurable neurophysiological changes using the selected metrics, making it difficult to establish causal relationships. This underscores that the underlying neural mechanisms of stroke recovery remain poorly understood, and multiple hypotheses may shed light on the neuroplastic changes mediating the observed behavioral improvements following traditional rehabilitation and non-invasive neuromodulation interventions.

Although cognitive effects were not the primary focus of any of the included studies, cognitive variables were assessed in six studies, with three of them showing significant improvements based on measures such as the MMSE, sRT, and cRT (107,108,127). The remaining three studies did not report cognitive improvements (114,117,124).

One of the primary limitations of this review stems from the variability observed in rTMS procedures, including variations in the duration and intensity of treatment, as well as differences in outcome measures and control intervention protocols. Additionally, the duration of follow-up in the studies reviewed varied. This variability prevented the aggregation of data for quantitative analyses, representing a significant limitation in this review.

Furthermore, it should be noted that none of the studies included in this review reported on the associated costs or potential economic benefits of rTMS interventions, as such considerations were not their primary focus. Consequently, there exists a notable gap in our current understanding regarding the cost-effectiveness of rTMS interventions.

3.2. OBJECTIVE 2: TO DESIGN A CLINICAL TRIAL TO STUDY THE CLINICAL IMPACT OF COMBINING MI-BASED NFB TRAINING, FOLLOWING BILATERAL RTMS NEUROMODULATION IN COMPARISON TO BILATERAL RTMS ALONE FOR THE REHABILITATION OF UPPER LIMB MOTOR SEQUELAE AFTER SUFFERING A STROKE WITH MORE THAN 3 MONTHS OF EVOLUTION.

3.2.1. STUDY DESIGN AND PARTICIPANTS

The SPIRIT 2013 Checklist has been used to assure the quality of the protocol (205). Participants were recruited from the Brain Injury Unit or Rehabilitation Unit of Beata María Ana Hospital. Additionally, patients referred from other medical centers and self-referred patients were also considered. A comprehensive assessment of potential subjects was conducted by a neurologist and a physiotherapist.

All patients met specific criteria, including a diagnosis of hemispheric ischemic or hemorrhagic stroke (>3 months after stroke) confirmed by at least one brain-imaging test and the presence of motor sequelae in the upper limb. The first three months after the stroke were excluded from consideration due to the phenomenon known as "spontaneous recovery" (206), which occurs during this period. Therefore, it would have been challenging to attribute clinical improvements solely to the intervention.

Patients included in the study exhibited impaired mobility and/or functionality in the UL because of the stroke. Further specific inclusion and exclusion criteria are detailed elsewhere (table 4):

Table 4. Participant selection criteria.

INCLUSION CRITERIA	EXCLUSION CRITERIA
Older than 18 years old.	History of seizure or brain aneurysm
Ischemic or hemorrhagic cerebrovascular injury diagnosed by a neurologist and who have at least one brain-imaging test	Pacemakers, medication pumps, metal implants in the head (except dental implants)
Onset of hemispheric ischemic or hemorrhagic stroke >3 months	Clinical instability
Kinesthetic and Visual Imagery Questionnaire (KVIQ) >55.	Muscle tone in the wrist and hand with a modified Ashworth scale (MAS) score equal to or higher than 3 in the wrist
Stability in antispastic medication for more than 5 days	Other pre-existing neurological diseases or previous cerebrovascular accidents with sequelae
Able to read and write	Aphasia
Sufficient cognitive ability to understand and perform tasks: Token Test >11	Previous TMS after stroke Hemispatial neglect (Bells Test >6 omissions on one side) Visual problems
	Flaccid paralysis Brunnstrom's stage = 1

The study was designed as a randomized, single-blind, controlled clinical trial, with patients randomly allocated to two groups:

1. Conventional rehabilitation + bilateral rTMS + Immersive multimodal BCI-VR training system NeuRow (NeuroRehabLab, Funchal, Portugal).
2. Conventional rehabilitation + bilateral rTMS.

For promoting adherence, any missed sessions within the established limit would be rescheduled for the following week. Flexible therapy schedules were offered, and patients' families were directly contacted by phone to confirm evaluation dates, thereby enhancing treatment adherence (207).

To calculate the sample size, the GRANMO calculator was employed, accepting an alpha risk of 0.005 and a beta risk of 0.2 in a one-sided contrast. This analysis determined that 23 subjects were required to detect a difference equal to 4.9 points, which represents the minimal clinically important difference in the Fugl-Meyer assessment scores for wrist/hand (208). Accounting for a 15% potential loss to follow-up, a sample size of 21 patients was targeted for each group.

Randomization and blinding procedures were conducted using the Research Randomizer application (Social Psychology Network, Middletown, CT, USA) (209), which assigned patients to groups using a two-digit code (1 and 2). The application generated a random list of 30 numbers containing the digits 1 and 2. This randomization process was performed remotely by a blinded researcher not involved in other research activities. Following randomization, another blinded staff member assigned patients to their respective groups. Allocation concealment was ensured using sealed, sequentially numbered envelopes. Evaluators received patients in separate rooms, unaware of the group to which each patient belonged.

To monitor adverse effects, participants were queried at the end of each session regarding any sensations experienced, such as tingling, burning, headache, drowsiness, among others, along with the intensity of these sensations. Safety guidelines for rTMS protocols were adhered to (210), with no expectation of severe adverse effects. Any adverse effects were to be reported to a licensed medical doctor, and management of possible adverse effects was to be individualized and based on their severity.

In terms of data processing security, data was recorded separately and underwent anonymization and storage in accordance with current European data protection laws. A specialized database designed for the study recorded and verified all data twice.

3.2.2. INTERVENTION PROTOCOLS

The two intervention protocols have been drawn from previous publications that demonstrated successful application in UL rehabilitation. On one hand, the study by Takeuchi et al. revealed significant improvements in subjects who received bilateral rTMS stimulation, based on the principle of interhemispheric inhibition (121). On the other hand, Vourvopoulos et al. published a pilot study using NeuRow, which demonstrated high performance (211).

The intervention involved the sequential administration of these two therapies on the same day, with rTMS application preceding the MI-based NFB training. In the control group, only rTMS application will be administered. The decision regarding the sequence of administration was based on preliminary results from the NeuroMOD project (unpublished) conducted by our group. These results indicated that patients who received rTMS prior to NFB exhibited better performance.

3.2.2.1. rTMS STIMULATION

Initially, single-pulse TMS was performed to determine the resting motor threshold (RMT). A Magstim Rapid2 device from Magstim Company, Whitland, Wales, UK, equipped with an air-cooled 70mm figure-of-eight magnetic stimulator coil, was utilized. For all assessments, the magnetic stimulator coil was placed over the primary motor cortex (M1) in the hemisphere under evaluation. Simultaneously, surface electromyography (EMG) recordings were collected from the contralateral first dorsal interosseous muscle using the CED Signal Software (CED, Cambridge, UK). EMG data were recorded and stored on a computer for subsequent offline analysis. The stimulation output intensity required to establish the RMT was used to calculate the parameters for the stimulation sessions.

The rTMS parameters were consistent with those described by Takeuchi et al. (121): The stimulation was set at 90% of the RMT, administered at a frequency of 1 Hz, with a total of 1000 pulses. This involved a 50-second inter-train interval applied to M1 in the hemisphere unaffected by the stroke. After a 5-minute rest period, stimulation of the contralateral M1 area took place at 90% of the RMT, with a frequency of 10 Hz and a total of 1000 pulses, utilizing a 5-second inter-train interval. These procedures were conducted over ten consecutive daily sessions from Monday to Friday.

3.2.2.2. MOTOR IMAGERY BASED NEUROFEEDBACK TRAINING

The NeuRow system is a gamified BCI training paradigm in virtual reality that enables patients to perform motor actions like those they would in real life. NeuRow is experienced through a head-mounted VR headset with a horizontal field of view of 90 degrees. Haptic feedback is delivered through two controllers held in both hands. During training, the paretic limb is placed in a resting position on the table.

Before setting up the EEG cap and VR headset for each session, a preliminary training was conducted with the following instructions:

Patients were asked to perform the rowing movement with both ULs with external facilitation of the paretic side.

Patients were instructed to imagine the movement with their eyes closed, focusing on their internal perspective and the sensation of rotation. They should visualize their hand closed in a fist and feel the weight and contraction of arm muscles. Patients should imagine the movement slowly and gradually increase its speed.

The most effective strategies for each participant were identified. Patients described in detail what they felt or tried to visualize, and the researcher provided feedback and described the sensation of the movement to the participant during the motor imagination, outlining the sequence of required movements (e.g., stretching the elbow, grasping the paddle, etc.).

The training was conducted daily and preceded the application of the NeuRow system. EEG acquisition was carried out before the VR training, using a BCIs system with 64 active electrodes, a low-noise biosignal amplifier, and a 24-bit A/D converter at 256 Hz (BrainVision actiCHamp biosignal amplifier, Brain Products GmbH, Gilching, Germany). The electrodes were primarily distributed over the motor and somatosensory areas of the brain, covering the frontal (F3, Fz, F4), frontal-central (FC5, FC6), central (C3, Cz, C4), central-parietal (CP5, CP1, CP2, CP6), and parietal (P3, Pz, P4) regions in a small Laplacian configuration for spatial filtering (figure 16).

EEG data acquisition and processing were conducted using the OpenVibe platform (212), which transmitted the data via the Lab Streaming Layer (LSL) protocol to control the virtual environment.

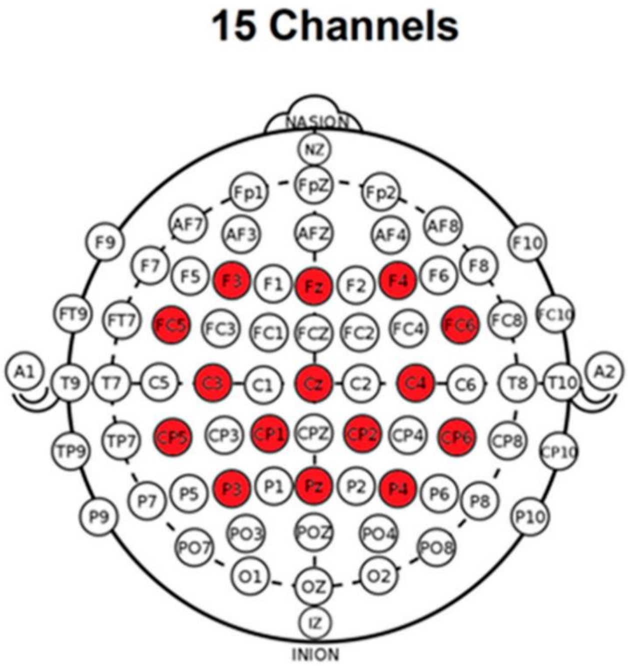

Figure 16. The spatial distribution of the electrodes. In red, the active electrodes.

Subsequently, a BCI training protocol was implemented based on the Graz-BCI paradigm (213). The initial step involved acquiring raw EEG data to extract features for training a classifier to distinguish between right and left imagined hand movements. Patients were instructed to perform mental imagery of the corresponding hand based on stimuli presented on the screen. The training session consisted of acquiring data in 24 blocks per class (right- or left-hand imagery) in a randomized order.

Following data acquisition, the data was filtered both spatially and temporally between the alpha and beta bands (8–30Hz) to create the feature vector.

During the training session, patients wore a VR headset and observed a boat with two high-fidelity virtual arms gripping two oars from a first-person view. They were required to imagine the movement of each corresponding hand to rotate each oar and make progress, all while observing the imagined movement on the screen (180). The game interface included timekeeping and scoring, with the goal of completing as many correct Motor Imagery sequences as possible within a fixed amount of time. To enhance adherence, points were awarded based on performance in each session (figure 17) (214).

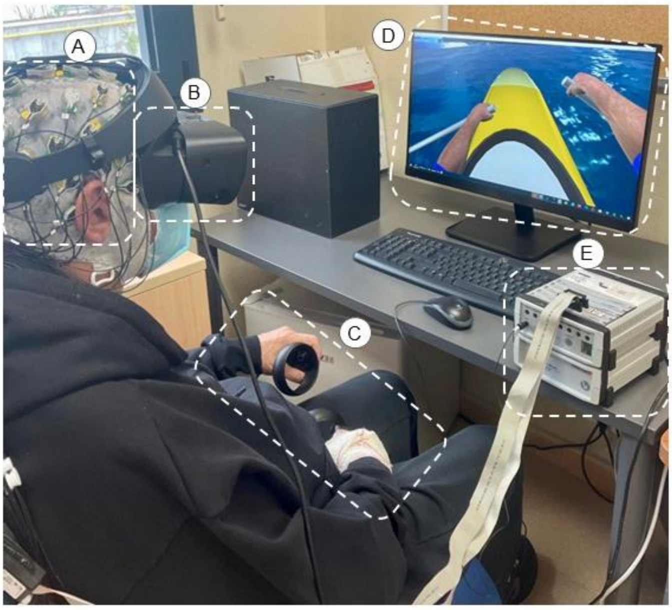

Figure 1715.Experimental Setup: (A) EEG cap with 64 active electrodes; (B) VR Head-Mounted Display; (C) Vibrotactile feedback; (D) VR Training Task; (E) EEG amplifier.

The MI training mode was employed, and training sessions were conducted over 12 non-consecutive sessions (on Mondays, Wednesdays, and Fridays) spanning four weeks. Each session lasted 45 minutes and was divided into three series of 7 minutes each, which included initial training and break time to prevent fatigue.

3.2.3. OUTCOMES MEASUREMENT

Three evaluations were conducted for each patient: a pre-intervention initial evaluation, a second evaluation the week following the end of the rTMS intervention, and a final evaluation after two weeks, upon completion of the NeuRow training. The first and third evaluation sessions had an average duration of 120 minutes, while the second evaluation lasted 60 minutes (figure 18).

3.2.3.1. PRIMARY OUTCOMES:

- Motricity Index of the Arm (MI): This assessment measures muscle strength in three muscle groups of the affected arm: grip, elbow flexion, and shoulder separation. Each movement is scored discretely, with scores ranging from 0 (no movement) to 33 (normal), resulting in a total score for the UL ranging from 0 (severely affected) to 100 (normal). This assessment method is widely used in rehabilitation progress evaluation and has a normalized and weighted scoring system (215,216).

- Dynamometry: Isometric grip strength was assessed using a handheld analogic dynamometer (Jamar® Plus+ Hand Dynamometer, 0–90kg). Patients performed a maximal isometric grip contraction, and three measures were taken with 1-minute rest between tests. They performed this task with both arms. The mean value was recorded to provide an objective evaluation of hand grip strength for pre- and post-protocol comparisons.

- Fugl-Meyer Assessment for Upper Limb (FMA-UL): This observational rating scale assesses sensorimotor impairments in post-stroke patients and includes four subscales: Upper Extremity, Wrist, Hand, and Coordination/Speed, with a total maximum score of 66 points. The FMA-UE is considered suitable for detecting changes in motor recovery in stroke patients, with a minimum clinically important difference (MCID) determined for different regions of the UL (217,218).

- Stroke Impact Scale (SIS): The SIS is a stroke-specific quality of life instrument that assesses the quality-of-life impairment after stroke, focusing on the physical domain. It includes four subscales, and in this study, only the hand function domain will be evaluated (219).

3.2.3.2. SECONDARY OUTCOMES:

- Computerized Finger Tapping Task (FTT): This task measures motor function and is sensitive to the slowing down of responses. Patients were instructed to repeatedly press the spacebar on a computer keyboard as fast as possible with their non affected index finger. The average time between two consecutive taps in five trials was recorded.

- Nine Hole Peg Test (NHPT): The NHPT evaluates UL dexterity. Patients are required to pick up nine pegs one by one from a container, transfer them into a target pegboard with nine holes, and then return them to the container. The time spent to complete the entire task with the affected arm is the outcome variable (220,221).

- Modified Ashworth Scale (MAS): This scale assesses spasticity and is one of the most used tools for its assessment. It will be employed to detect potential secondary changes in spasticity if motor changes are produced (222,223).

- Nottingham Sensory Assessment (NSA): This assessment evaluates somatosensory impairment of the UL, which occurs in approximately 50% of adults after a stroke. It provides a measurement of sensory impairment and may reveal changes related to sensitivity after the intervention with the NeuRow system (224–226).

- Barthel Index (BI): This index assesses activities of daily living (ADLs) in stroke patients and has been widely used for this purpose (227,228).

- Neurophysiological measurements of cortical plasticity changes: This includes assessments such as TMS Resting Motor Threshold (RMT) in the first dorsal interosseous muscle or the abductor pollicis brevis muscle to determine cortical excitability changes and their correlation with clinical outcomes.

- EEG: Various measures of quantitative EEG were collected to evaluate stroke patients' recovery (229).

These assessments provided a comprehensive evaluation of the impact of the intervention on motor function, quality of life, and neurophysiological changes in stroke patients.

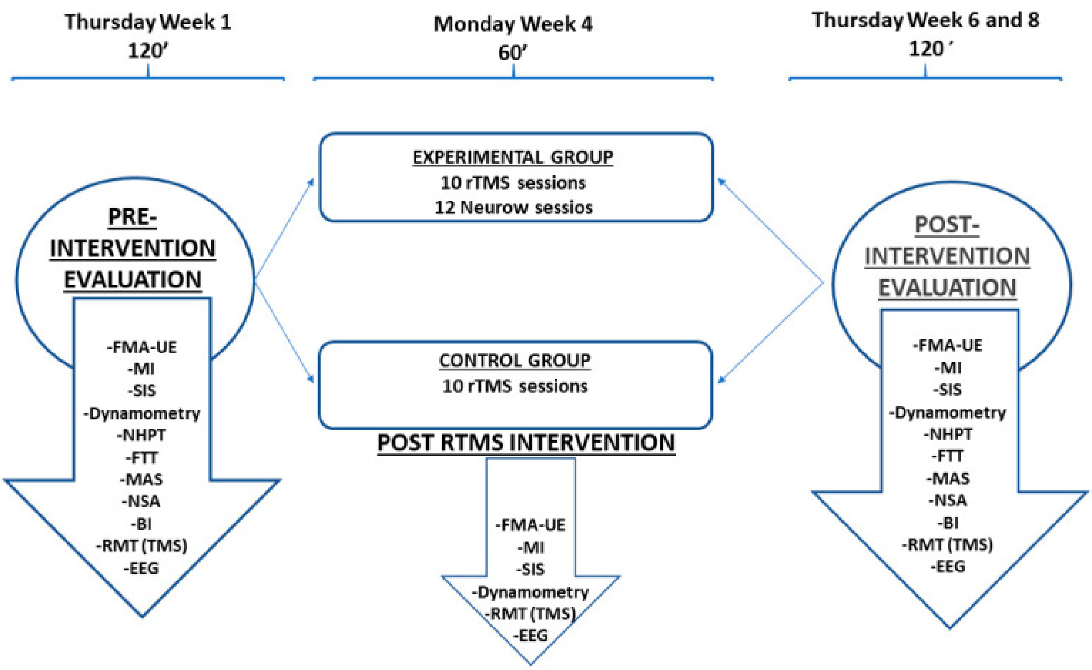

Figure 1816. Schematic representation of intervention protocol. It includes the days of the week and duration of each block of outcomes measurement in pre- and post-evaluation.

3.2.3. DISCUSSION

NIBS, involving repetitive activation of specific brain regions using magnetic stimulation, holds significant promise as a complementary therapeutic approach alongside traditional post-stroke physical therapy (230). Some rTMS (repetitive transcranial magnetic stimulation) protocols, which aim to address interhemispheric inhibition compensation, have been documented as effective in reinforcing neuroplastic changes (193).

In contrast, studies conducted by Vourvopoulos et al. (180) and Ramos-Murguialday et al. (231), utilizing multimodal immersive brain-computer interface-virtual reality (BCI-VR) training, have also demonstrated enhancements in the rehabilitation process's carry-over effect. This improvement was evident through clinical assessments, self-reported evaluations, electrophysiological measurements, and neuroimaging data.

Previously, several investigations have successfully combined different non-invasive neuromodulation approaches in stroke patient rehabilitation (232–234). However, this marks the first instance of evaluating multimodal immersive BCI-VR training as an enhancer for the proven effectiveness of rTMS bilateral protocols targeting interhemispheric inhibition compensation.

In the context of post-stroke hand rehabilitation, it appears that cortico-subcortical connectivity mechanisms play a pivotal role (235). Exogenous neuromodulation via rTMS represents a top-down cortical excitability input, stimulating both the cortex and cortico-spinal pathway. Conversely, endogenous neuromodulation, such as motor imagery, may parallel bottom-up rehabilitation mechanisms have triggered by physical motor engagement, thereby activating cortical and subcortical mechanisms (236). By combining these two therapies, it is hypothesized that cortico-subcortical connectivity will be further enhanced, leading to greater motor recovery of the affected limb.

The integration of these techniques, along with a thorough and objective evaluation of UL outcomes, will contribute to a novel hypothesis regarding how different neuromodulation approaches can impact homeostatic plasticity and, consequently, motor recovery. The validation of this protocol will ascertain the clinical utility of combining two non-invasive neuromodulation methods to augment conventional stroke rehabilitation. This study's findings will help identify whether multimodal immersive BCI-VR training amplifies the established effects of rTMS on interhemispheric inhibition. Additionally, neurophysiological, and clinical factors predicting responses to this protocol.

3.3. OBJECTIVE 3: TO EXAMINE THE CLINICAL EFFECTS INDUCED BY COMBINING MI-BASED NFB TRAINING WITH BILATERAL REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION FOR UPPER LIMB MOTOR FUNCTION IN LATE SUBACUTE AND CHRONIC STROKE PATIENTS.

3.3.1. STUDY DESIGN AND PARTICIPANT

A clinical trial utilizing an AB/BA crossover design was conducted in accordance with the CONSORT 2010 guidelines (237).

The protocol had some changes in its design due to economic constraints impeding a full recruitment and some variables were also changed to assure a more comprehensive evaluation of the functional effects of the therapy.

The updated trial protocol was prospectively registered on clinicaltrials.gov with the unique identifier NCT04815486. Ethical approval was obtained from an independent Clinical Research Ethics Committee, and the study adhered to the principles outlined in the Declaration of Helsinki of 1964, with the latest revision in 2013. All participants provided written informed consent before their inclusion.

Twenty consecutive individuals who met the eligibility criteria successfully completed the assigned interventions. For a visual representation of participant flow, refer to Figure 21 in the results section. Recruitment primarily took place at a hospital in Madrid, Spain, with patients coming from the Brain Injury Unit or Rehabilitation Unit. Participants referred from other medical centers and self-referred individuals were also enrolled.

3.3.1.1. ELIGIBILITY CRITERIA

INCLUSION CRITERIA

- Age greater than 18 years.
- Diagnosis of ischemic or hemorrhagic cerebrovascular injury by a neurologist, with at least one brain-imaging test.
- Onset of symptoms more than 3 months prior to enrollment.
- Presence of UL motor sequelae resulting from a stroke.
- Stability in antispastic medication for at least 5 days.
- Ability to read and write.

- Sufficient cognitive capacity to comprehend and perform tasks, with a Token test score greater than 11 (238).

EXCLUSION CRITERIA:

- History of seizures or brain aneurysms.
- Presence of pacemakers, medication pumps, or metal implants in the head (excluding dental implants).
- Clinical instability.
- Aphasia.
- Pre-existing neurological conditions or previous cerebrovascular accidents with sequelae.
- Prior rTMS interventions received after a stroke.
- Hemispatial neglect.
- Flaccid paralysis with a Brunnstrom's stage less than 1 (239).

3.3.2. INTERVENTIONS

3.3.2.1. BILATERAL RTMS

The bilateral rTMS protocol was applied based on the method described by Takeuchi et al. in 2009 (19). Initially, single-pulse TMS was administered to determine the resting motor threshold (RMT) using a Magstim Rapid2 device (Magstim Company, Whitland, UK) equipped with a 70 mm figure-of-eight coil. The coil was positioned on the primary motor cortex (M1) contralateral to the stroke lesion. RMT was calculated using established methods (240).

The rTMS intervention comprised 10 consecutive daily sessions over a span of two weeks (Monday to Friday). Each session involved stimulation of both M1 regions. It began with low-frequency (1 Hz) stimulation over the unaffected side, followed by a resting period of 5 minutes, and then high-frequency (10 Hz) stimulation over the lesioned side. Each hemisphere received a total of 1000 pulses, at an intensity of 90% RMT (or a maximum of 52% of the Magstim Rapid2 device's default maximum output, as per its safety system). The pulses were distributed into 20 trains, each containing 50 pulses, with a 5-second intertrain interval (121).

Participants were queried at the conclusion of each session regarding any tingling sensations, headaches, neck pain, drowsiness, and the intensity of these sensations to assess side effects. Safety protocols associated with rTMS procedures were strictly adhered to (92).

3.2.2.2. MOTOR IMAGERY-BASED NEUROFEEDBACK

NeuRow is a MI-NFB training paradigm that enables patients to perform UL motor actions as they would in real life. It incorporates a BCI system rooted in MI and guided by NFB using EEG.

NeuRow is delivered through a head-mounted VR headset, offering a horizontal field of view of 90 degrees. Haptic feedback is provided via two controllers held in both hands. The paretic limb is positioned in a resting state on a table. After ensuring the patient's position and UL alignment are correct, the EEG cap and VR headset are configured.

The task involves mentally simulating unilateral rowing movements with each UL alternately. In the virtual environment, patients visualize a boat and two high-resolution virtual arms grasping two oars, presented from a first-person perspective. Patients must imagine the movement of each corresponding hand to turn each oar and propel the boat forward, while observing the imagined movement on the screen. The objective is to execute as many accurate motor image sequences as possible within a fixed time frame.

EEG data is collected using a BCI system equipped with 64 active electrodes, a low-noise biosignal amplifier, and a 256 Hz 24-bit A/D converter (BrainVision actiCHamp biosignal amplifier, Brain Products GmbH, Gilching, Germany). EEG data acquisition adheres to the international 10-20 system for electrode placement. A total of 15 electrodes for BCI are strategically located, primarily covering the motor and somatosensory areas of the brain. These electrodes include those in the Frontal (F3, Fz, F4), Frontal-Central (FC5, FC6), Central (C3, Cz, C4), Central-Parietal (CP5, CP1, CP2, CP6), and Parietal (P3, Pz, P4) regions, configured in a small Laplacian arrangement to facilitate spatial filtering. Both EEG data processing and acquisition are conducted through the OpenVibe platform (212), transmitting data via the Lab Streaming Layer protocol to control the virtual environment.

A BCI training protocol, adapted from the Graz-BCI paradigm (213), is employed. Initially, raw EEG data is collected, and features are extracted to train a classifier capable of distinguishing between mental images of right and left-hand movements. Patients are instructed to create mental images corresponding to the hand indicated by stimuli on the screen. The training session comprises 24 blocks per class (left- or right-hand images) presented in random order. Subsequently, the data is filtered both spatially and temporally, focusing on the Alpha and Beta bands (8–30 Hz) to generate the feature vector (figure 19).

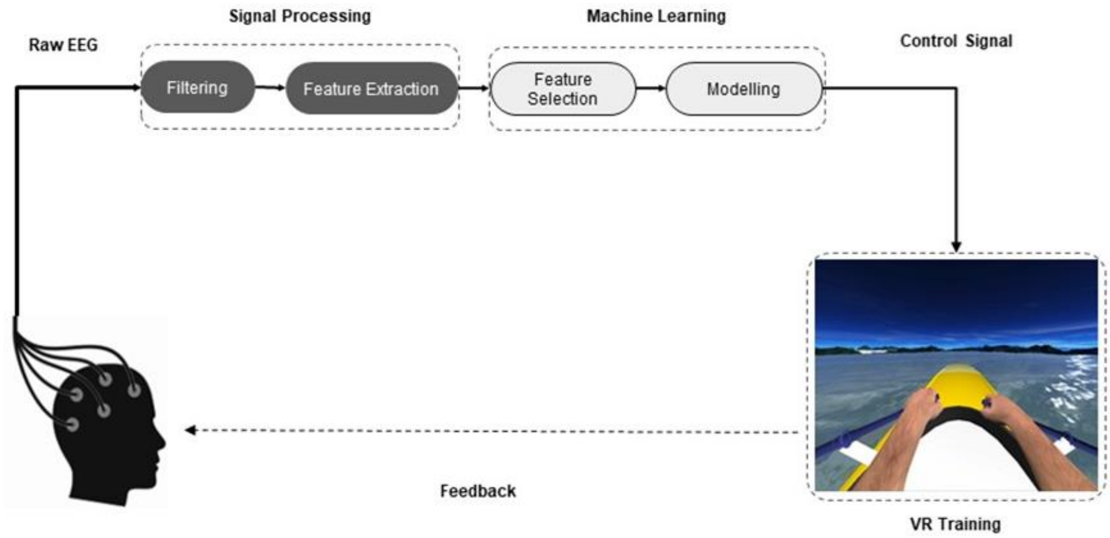

Figure 1917. Motor Imagery-based Neurofeedback with immersive Virtual Reality (VR) protocol.

Training is conducted over 12 sessions, with sessions held three days a week for four weeks, each lasting 30 minutes. These sessions are divided into three series, each spanning 7 minutes (180).

3.2.2.3. EXPERIMENTAL PROCEDURE

The interventions are structured as follows: Therapy A exclusively entails the bilateral rTMS protocol, consisting of 10 consecutive daily sessions over two weeks. In contrast, Therapy B combines the bilateral rTMS protocol with MI-NFB training. During Therapy B, patients receive 10 consecutive daily sessions of bilateral rTMS (Monday to Friday, two weeks) with the same stimulation parameters as Therapy A. Additionally, they undergo 12 non-consecutive sessions of MI-neurofeedback (three times a week for four weeks). The first 6 MI-NFB sessions are administered following bilateral rTMS (i.e., rTMS serves as a priming method during the initial two weeks), while the remaining 6 sessions are conducted without prior rTMS during the latter two weeks.

The clinical trial follows an AB/BA crossover design with a counterbalanced assignment. The first 50% of the sample is assigned to receive therapies in the order of AB, while the second 50% is assigned to receive them in the reverse order of BA. A one-month washout period is consistently applied between the two therapies (Figure 20).

Participants undergo assessment at six different time points (M1, M2, M3, M4, M5, and M6), with three assessments completed for each therapy. These assessments include an initial evaluation prior to the intervention, a second evaluation one week after the intervention's conclusion, and a final evaluation two weeks following the conclusion (Figure 20).

Throughout their participation in the study, patients continue with their regular therapies, with no changes in therapy dosage or content made by the research team. These therapies are not controlled or influenced by the research team.

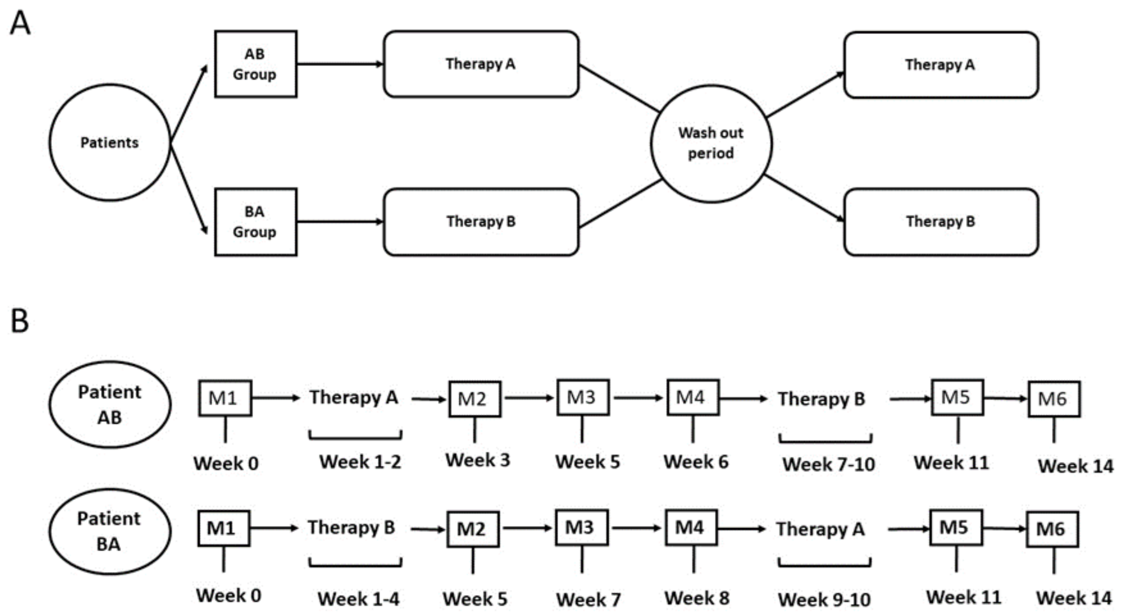

Figure 18.Experimental design. A. Therapy sequence in each group where Therapy A is repetitive transcranial magnetic stimulation alone and Therapy B is repetitive transcranial magnetic stimulation plus Motor Imagery-based Neurofeedback training. B.

3.2.2.4. OUTCOME MEASURES

3.2.3.4.1. PRIMARY OUTCOME MEASURES

- <u>Fugl-Meyer Upper Limb Assessment (FMA-UL):</u> This observational scale assesses sensorimotor deficits in post-stroke patients, comprising four subscales with a total score of 66 points. It is considered the optimal and sensitive scale for evaluating UL functionality (217,218).

- Hand Grip Strength: Isometric grip strength is assessed using an analog hand dynamometer (Jamar® Plus+ Hand Dynamometer, 0–90 kg). Patients are seated upright, with both feet on the floor and their forearm resting on a stable surface. They perform a maximal isometric grip contraction, and the mean value of three attempts per hand is recorded (241).

- Nottingham Sensory Assessment (NSA): This tool quantitatively measures somatosensory impairment in the UL. It evaluates tactile sensation, kinesthetic sensation, and stereognosis (225,226).

3.2.3.4.2. SECONDARY OUTCOME MEASURES

- Nine Hole Peg Test (9-HPT): Fine manual dexterity is assessed using this test. Patients are tasked with quickly transferring nine pegs, one at a time, from a container to a pegboard with nine holes. This test is conducted with both hands, and the time taken to complete the task is recorded (30). The 9-HPT is recognized as a reliable, valid, and sensitive tool for detecting changes in stroke patients (217,221).

- Computerized Finger Tapping Task (FTT): This test measures motor function and signal processing speed. Patients sit comfortably in front of a computer and repeatedly press the space bar on the keyboard as quickly as possible with their index finger. Five attempts, each lasting 10 seconds, are made with the unaffected hand, and the average time between consecutive touches in the five attempts is recorded (242).

- Arm Motricity Index (AMI): This discrete quantitative scale evaluates muscle strength in three actions: pinch, elbow flexion, and shoulder separation. Each movement is scored, yielding a total score for the upper limb, ranging from 0 (severely affected) to 100 (normal) (215,216).

- Modified Asworth Scale (MAS): A clinical scale used to measure the degree of spasticity in patients with neurological impairments, particularly in conditions such as stroke. It assesses resistance to passive movement in joints, assigning scores from 0 to 4 (0 = no spasticity, 4 = rigid muscle contraction). It is a subjective and widely used measure but may have limitations in accuracy due to its focus on movement resistance. We measure the degree of spasticity in both elbow flexion and wrist flexion (243).

- Modified Barthel Index (MBI): A widely used functional assessment scale to gauge independence in activities of daily living among individuals with physical disabilities or neurological conditions like stroke. It evaluates an individual's capacity to perform 10 basic activities such as feeding, dressing, controlling bowel and bladder functions, among others. The total score can range from 0 (total dependence) to 100 (total independence) (244).

- Stroke Impact Scale (SIS-16): It is a shortened version of the Stroke Impact Scale consisting of 16 items assessing an individual's perception of their health status post-stroke across physical, emotional, and social domains. Each item is scored on a scale from 0 to 5, with the total scores ranging from 0 to 80. Higher scores reflect better perceived health and a reduced impact of the stroke on the individual's life (245).

3.2.2.5. STATISTICAL ANALYSIS

Statistical analysis was conducted using SPSS 26.0 (SPSS Inc., Chicago, USA) and JASP 0.17.2 (JASP Team 2023). All analyses adhered to a confidence level of 0.95 (alpha = 0.05), with 95% confidence intervals obtained. Prior to hypothesis testing, normality was verified using the Shapiro-Wilk test, and equality of variances between groups was assessed using Levene's test.

To begin, the presence of carry-over effects was assessed through repeated measures ANOVA. Subsequently, therapy effects were analyzed using a linear mixed-effects model (LMM) with therapy, time, and sequence (AB/BA) as fixed-effects, and participants as random-effects.

3.3.3. RESULTS

3.3.3.1. PARTICIPANT CHARACTERISTICS

Participant flow can be reviewed in Figure 21. Out of the initial 23 volunteers screened, 20 participants successfully completed the study and were subsequently analyzed. Baseline characteristics of the participants are summarized in Table 5 and demonstrated no statistically significant differences between groups (P>0.05). Statistical tests, including the Shapiro-Wilk normality test and Levene's test, did not reveal significant differences in the outcome measures used (P>0.05).

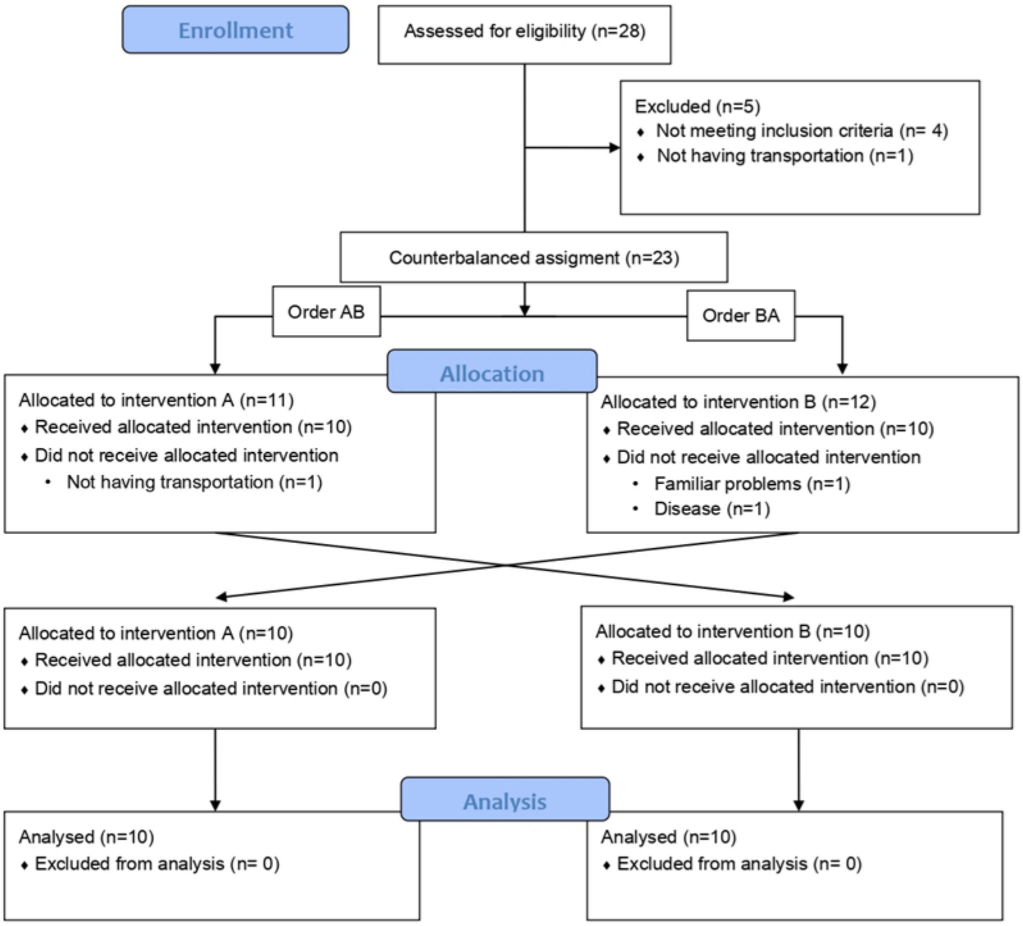

Figure 21. Participant flow diagram according to CONSORT 2010 guidelines.

Table 5. Demographic and clinical characteristics at baseline.

Characteristics	AB Group (N=10)	BA Group (N=10)	P-value
Age, years, mean (SD)	68.50 (9.02)	62.30 (8.32)	0.128
Sex, n (%)			0.371
Female	6 (60%)	4 (40%)	
Male	4 (40%)	6 (60%)	
Time since stroke, months, mean (SD)	27 (21.01)	27.70 (35.51)	0.958
Stroke type, n (%)			0.606
Ischemic	7 (70%)	8 (80%)	
Hemorrhagic	3 (30%)	2 (20%)	
Affected structure, n (%)			0.541
Cortical	6 (60%)	7 (70%)	
Subcortical	3 (30%)	3 (30%)	
Dominance, n (%)			0.305
Right	10 (100%)	9 (90%)	
Left	0 (0%)	1 (10%)	
Affected hemisphere, n (%)			0.178
Right	3 (30%)	6 (60%)	
Left	7 (70%)	4 (40%)	
MoCA, mean (SD)	24.16 (5.08)	23.40 (3.97)	0.115
FMA-UL, mean (SD)	24.50 (16.21)	24.50 (11.84)	0.513
Hand-grip strength, Kgs, mean (SD)	4.03 (4.34)	4.87 (4.73)	0.677
9-HPT, seconds, mean (SD)	167.39 (94.92)	180.75 (103.76)	0.626
Arm MI, mean (SD)	42.10 (21.60)	42.40 (22.64)	0.852
FTT, second, mean (SD)	586.05 (802.16)	1921.54 (2803.82)	0.206
NSA-TS, mean (SD)	1.49 (0.57)	1.34 (0.36)	0.947
NSA-KS, mean (SD)	1.30 (0.53)	1.75 (0.48)	0.051
NSA-S, mean (SD)	4.49 (3.89)	5.16 (4.05)	0.674
MAS (Elbow-Flexion), mean (SD)	1.70 (0.94)	1.50 (0.76)	0.079
MAS (Wrist-Flexion), mean (SD)	1.30 (0.67)	1.88 (0.99)	0.094
MBI, mean (SD)	68.00 (22.87)	70.63 (26.10)	0.188
SIS, mean (SD)	53.90 (14.34)	52.75 (17.89)	0.654

Abbreviations: Arm MI: Arm Motricity Index; MoCA: Montreal Cognitive Assessment; FMA-UL: Fugl Meyer Assessment-Upper Limb; FTT: Finger Tapping Test; MAS_ Modified Ashworth Scale; MBI: Modified Barthel Index; 9-HPT: Nine Hole Peg Test; NSA-TS: Nottingham Sensory Assessment-Tactile Sensations; NSA-KS: Nottingham Sensory Assessment-Kinesthetic Sensations; NSA-S: Nottingham Sensory Assessment-Stereognosis, SIS: Stroke Impact Scale and SD: Standard Deviation.

Table 6. Values of outcome measures by time and sequence of treatment.

Variable	Measurement					
	1	**2**	**3**	**4**	**5**	**6**
FMA-UL, mean (SD)						
AB	24.50 (16.21)	29.20 (18.13)	29.20 (17.35)	28.80 (16.59)	36.50 (16.41)	36.70 (16.64)
BA	24.50 (11.84)	42.50 (18.51)	42.50 (19.50)	42.20 (19.16)	43.50 (18.78)	42.90 (20.53)
Hand-grip strength, Kgs, mean (SD)						
AB	4.03 (4.34)	4.47 (4.31)	4.16 (4.37)	4.03 (4.10)	5.18 (4.74)	5.04 (4.37)
BA	4.87 (4.73)	9.10 (10.47)	7.18 (6.13)	6.54 (6.11)	7.13 (6.21)	7.44 (7.39)
NSA-TS, mean (SD)						
AB	1.49 (0.57)	1.45 (0.60)	1.36 (0.57)	1.30 (0.55)	1.77 (0.48)	1.76 (0.42)
BA	1.34 (0.36)	1.76 (0.38)	1.74 (0.39)	1.77 (0.69)	1.66 (0.59)	1.70 (0.50)
NSA-KS, mean (SD)						
AB	1.30 (0.53)	1.35 (0.47)	1.35 (0.47)	1.22 (0.47)	2.12 (0.77)	1.98 (0.52)
BA	1.75 (0.48)	2.30 (0.42)	2.37 (0.56)	2.27 (0.55)	2.17 (0.60)	1.95 (0.43)
NSA-S, mean (SD)						
AB	4.49 (3.89)	4.50 (3.89)	4.40 (3.97)	4.40 (3.97)	4.63 (3.77)	4.53 (3.86)
BA	5.16 (4.05)	5.46 (3.72)	4.76 (3.64)	4.63 (3.76)	4.70 (3.71)	4.76 (3.64)
9-HPT, seconds, mean (SD)						
AB	167.39 (94.92)	149.23 (91.20)	137.52 (93.14)	153.02 (106.54)	138.86 (99.65)	124.64 (96.33)
BA	180.75 (103.76)	151.04 (81.72)	167.72 (103.40)	171.71 (139.92)	146.27 (121.17)	107.75 (91.84)
AMI, mean (SD)						
AB	42.10 (21.60)	50.80 (22.30)	48.50 (25.35)	40.40 (24.51)	65.70 (26.00)	64.10 (29.18)
BA	42.40 (22.64)	70.70 (23.99)	67.70 (29.22)	66.20 (28.06)	70.80 (23.60)	70.00 (24.50)
FTT, milliseconds, mean (SD)						
AB	586.05 (802.16)	520.14 (640.96)	633.13 (649.71)	708.34 (840.04)	2827.66 (6893.94)	670.66 (598.86)
BA	1921.54 (2803.82)	2734.07 (3700.31)	1738.35 (3156.39)	1536.39 (3076.39)	514.58 (464.81)	469.29 (674.84)
MAS (Elbow-flexion), mean (SD)						
AB	1.70 (0.94)	1.80 (0.79)	2.00 (0.81)	1.90 (0.87)	1.30 (0.67)	1.40 (0.84)
BA	1.50 (0.76)	1.25 (0.46)	1.25 (0.46)	1.38 (0.51)	1.25 (0.46)	1.50 (0.53)
MAS (Wrist-flexion), mean (SD)						
AB	1.30 (0.67)	1.10 (0.13)	1.10 (0.31)	1.10 (0.32)	1.10 (0.31)	1.10 (0.31)

BA	1.88 (0.99)	1.38 (0.74)	1.25 (0.70)	1.25 (0.70)	1.25 (0.70)	1.25 (0.46)
MBI, mean (SD)						
AB	68.00 (22.87)	70.00 (21.21)	70.00 (24.72)	70.00 (24.72)	76.00 (18.38)	75.50 (20.47)
BA	70.63 (26.10)	77.50 (23.75)	80.63 (24.41)	80,63 (24,55)	78.13 (24.33)	76.25 (22.63)
SIS, mean (SD)						
AB	53.90 (14.34)	54,10 (15.58)	57.70 (15.37)	57.80 (15.47)	58.10 (13.19)	56.50 (11.73)
BA	52.75 (17.89)	57.25 (16.10)	59.13 (17.55)	59.50 (18.12)	60.25 (18.21)	57.13 (16.12)

Abbreviations: Arm MI: Arm Motricity Index; FMA-UL: Fugl-Meyer Assessment-Upper Limb; FTT: Finger Tapping Test; MAS: Modified Asworth Scale; MBI: Modified Barthel Index; 9-HPT: Nine-Hole Peg Test; NSA-TS: Nottingham Sensory Assessment-Tactile Sensations; NSA-KS: Nottingham Sensory Assessment-Kinesthetic Sensations and NSA-S: Nottingham Sensory Assessment-Stereognosis; SIS: Stroke Impact Scale; SD: Standard Deviation

3.3.3.2. PRIMARY OUTCOME MEASURES

The results for each outcome measure and measurement time point are presented in Table 6.

- Fugl-Meyer UL Assessment (FMA-UL)

FMA-UL was the only variable that exhibited a significant carry-over effect (P = 0.03). According to the LMM analysis, significant effects were observed for the therapy factor on FMA-UL (F (1, 64) = 27.096; P < 0.001) and the time factor (F (5, 90) = 39.246; P < 0.001). No significant effects were identified for the sequence factor (F (1, 18) = 1.324; P > 0.05). However, a significant interaction between sequence and time was found (F (5, 90) = 9.164; P < 0.001). Post-hoc analyses demonstrated significant differences at measurement points 2 (E = 4.433; t = 5.385; P < 0.001), 3 (E = -2.217; t = -2.692; P = 0.008), 4 (E = -2.217; t = -2.692; P = 0.008), and 5 (E = -2.267; t = -2.753; P = 0.007) in favor of therapy B (Figure 22-A). The remaining measurements did not reveal significant differences (P > 0.05).

- Hand Grip Strength

The LMM analysis did not indicate significant outcomes for the therapy factor (F (1, 18) = 0.311; P > 0.05) or the sequence factor (F (1, 18) = 1.053; P > 0.05) in relation to Hand Grip Strength. However, significant effects were observed for the time factor (F (5, 90) = 3.127; P < 0.001). There was no significant interaction between sequence and time (F (5, 90) = 1.823; P > 0.05). Subsequent post-hoc analyses revealed significant differences at measurement points 2 (E = 0.862; t = 2.030; P = 0.045) and 3 (E = -1.036; t = -2.440; P = 0.017) in favor of therapy B (Figure 22-B). No significant variations were observed in the remaining measurements (P > 0.05).

- Nottingham Sensory Assessment (NSA)

Tactile Sensation (NSA-TS)

Significant effects were observed for the therapy factor (F (1, 18) = 7.065; P = 0.016) and the time factor (F (5, 90) = 7.130; P < 0.001) in relation to Tactile Sensation (NSA-TS). No significant effects were found for the sequence factor (F (1, 18) = 0.557; P > 0.05). A significant interaction effect was noted between sequence and time (F (5, 90) =

6.759; P < 0.001). Significant differences in favor of therapy B were observed at all measurement points (P < 0.05) (Figure 23-A).

Kinesthetic Sensation (NSA-KS)

Significant effects were observed for the therapy factor (F (1, 18) = 9.036; P = 0.004), the time factor (F (5, 90) = 11.146; P < 0.001), and the sequence factor (F (1, 18) = 7.647; P = 0.013) in relation to Kinesthetic Sensation (NSA-KS). A significant interaction effect was observed between sequence and time (F (5, 90) = 15.557; P < 0.001). Significant differences in favor of therapy B were noted at all measurement points except time 2 (E = 0.066; t = 1.156; P > 0.05) (Figure 23-B).

Stereognosis (NSA-S)

No significant effects were observed for the therapy factor (F (1, 18) = 0.636; P > 0.05), the time factor (F (5, 90) = 1.009; P > 0.05), the sequence factor (F (1, 18) = 0.063; P > 0.05), or the interaction between sequence and time (F (5, 90) = 0.905; P > 0.05) in relation to Stereognosis (NSA-S). There were no significant differences at any measurement points (P > 0.05) (Figure 23-C).

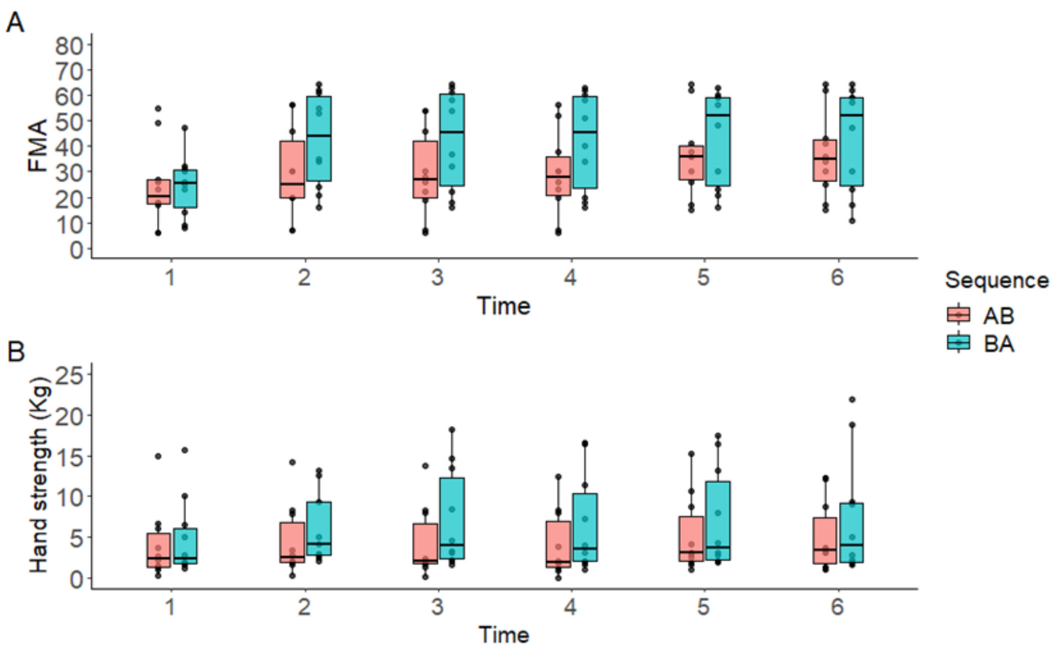

Figure 22. Changes in the Upper Limb Fugl-Meyer Assessment (FMA-UL) and Hand-Grip Strength over time, according to the treatment sequence.

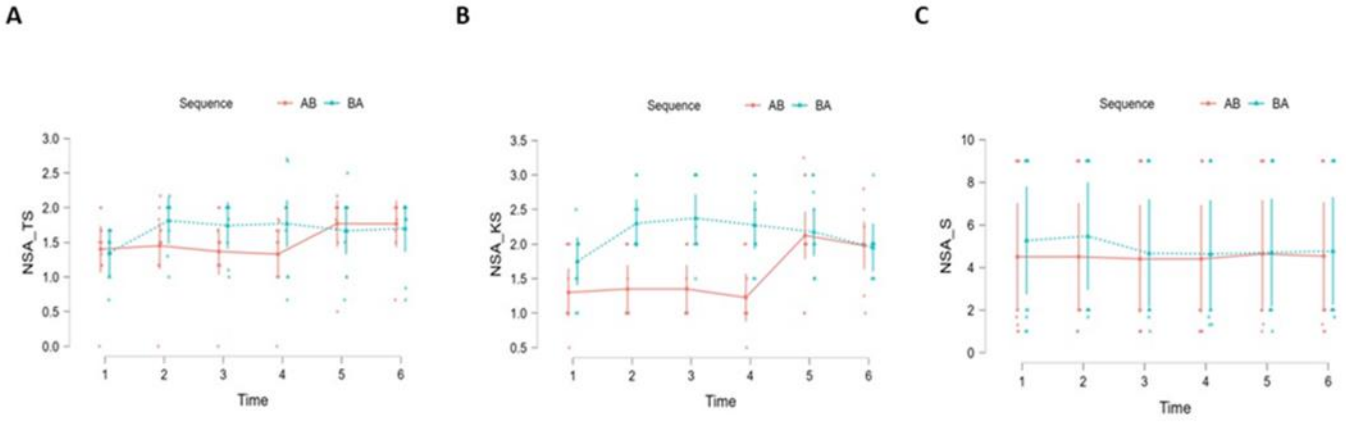

Figure 23. Changes in the Nottingham Sensory Assessment (NSA): A-Tactile Sensation (TS), B-Kinesthetic Sensation (KS) and C-Stereognosis (S) over time, according to the treatment sequence.

3.3.3.3. SECONDARY OUTCOME MEASURES

The therapy factor exhibited significant effects on the AMI (F (1, 93) = 13.021; P < 0.001). The sequence factor did not have a significant effect on the changes resulting from therapies in any outcome measure (P > 0.05). Both AMI and 9-HPT, as well as MAS for elbow flexion and MBI, demonstrated significant effects for the time factor (F (5, 90) = 17.556; P < 0.001), (F (5, 65) = 3.097; P = 0.014), (F (5, 80) = 3.519; P = 0.006), and (F (5, 90) = 3.654; P = 0.005), respectively. Regarding the interaction between the sequence and time factors, significant effects were found only in AMI (F (5, 90) = 4.949; P < 0.001). Only measurements 1 (E = 2.300; t = 2.953; P = 0.004) and 2 (E = -6550; t = -3120; P = 0.002) showed significant changes in favor of therapy B. No significant effects were observed for any of the factors in relation to the FTT.

3.3.4. DISCUSSION

In this study, we aimed to explore whether the combined application of bilateral rTMS and MI-based NFB training offers superior improvements in UL motor function for subacute and chronic stroke patients compared to rTMS alone. Our findings revealed a significant enhancement in UL motor function, as assessed by the FMA-UL, favoring the combined approach involving both rTMS and MI-based NFB. However, it's worth noting that contrary to our initial expectations, hand-grip strength did not exhibit any significant differences between the two therapeutic strategies. Additionally, concerning somatosensory deficits evaluated through the NSA, particularly in tactile and kinesthetic sensation, we observed notable improvements, again favoring the combined therapy, although no significant distinctions were found in stereognosis. Furthermore, the combined approach had a beneficial impact on secondary outcome measures, such as the AMI.Taken together, these results provide substantial support for the primary hypothesis of our study.

Interestingly, FMA-UL was the sole variable displaying a significant carry-over effect, implying that the effects of rTMS persisted beyond the intervention period. Given that all participants continued their conventional rehabilitation therapies during the washout month, it is conceivable that this concurrent rehabilitation might have influenced the sustained effects.

Our findings substantiate previous research demonstrating the advantages of combining rTMS and BCI training (106,188). However, our study not only affirms this concept in general but also offers a more comprehensive comprehension of the clinical efficacy of this specific combination. For instance, in a study by Johnsson et al. (106), a two-week combined protocol of rTMS and BCI training was compared to sham-rTMS in a cross-over design (n = 3). While their study differed from ours in terms of rTMS parameters and BCI training techniques, it still demonstrated significant improvements in gross manual dexterity (Box and Blocks test) in the sham rTMS+BCI condition and improvements in both conditions for the FTT test on the affected side, showcasing the beneficial effect of BCI with a clear enhancement by rTMS. Similarly, our study validates the superiority of the combination of both therapies based on the improvement of functional scales (FMA-UL), with the added benefit of observing effects on sensory variables.

In contrast, Chen et al. (188) utilized iTBS on the affected hemisphere as a primer for VR cycling training for the UL compared to sham iTBS as the priming for the same training. Although their study confirmed the beneficial effects of VR, it also revealed a clear enhancement resulting from iTBS priming, particularly evident in the Modified Ashworth Scale Upper-Extremity, Motor Activity Log, and Stroke Impact Scale. Unlike our study, Chen et al. (188) showed improvement in FMA-UL but did not demonstrate significant between-group differences.

Despite these methodological differences, both studies, along with ours, suggest that pre-training rTMS stimulation has the potential to modify or enhance the effects of NFB or VR. Our study further adds to this body of evidence by demonstrating that MI-based NFB enhances the effects of bilateral rTMS. Nonetheless, the optimal type of rTMS stimulation and the ideal combination therapy remain topics of ongoing debate. Bilateral rTMS application hinges on the combined neurophysiological effects stemming from both decreased excitability on the unaffected side (106) and increased excitability on the affected hemisphere (188).

Our study's observation of no substantial differences in hand-grip strength between the two therapies implies that both therapeutic strategies are equally effective in improving this metric. Notably, the NFB is grounded in imagined movement, which aligns with our findings in the NSA, where the combined approach exhibited significant effects on the touch and movement sensation tests, particularly positive changes in the distal part of the limb, improving both skills. This effectiveness can likely be attributed to the vibrating touch feedback and the embodiment provided by NeuRow (246,247).

Regarding secondary variables, we observed a significantly different effect between therapies only in the AMI and MAS for elbow flexion. This finding supports, in conjunction with the FMA-UL results, the clinical efficacy of the combined approaches in enhancing impaired limb functionality. The 9-HPT showed notable time effects, indicating that patients experienced significant improvements in fine manual dexterity after receiving both therapies, with no discernible differences between them. One potential explanation for the lack of an adjuvant effect of MI-based NFB could be a ceiling effect already achieved by rTMS stimulation. We hypothesize that functional brain changes for improving reaching movements involving the shoulder/elbow and distal motor control could rely on different stages of recovery or diverse neural plasticity patterns driving functional recovery (248). This may explain why enhancements in distal improvements in motor components were not observed with the combined treatment.

From the articles published thus far on this subject, only two studies include MBI, with differing results. Du et al. (104) did not find significant changes in this scale following rTMS application, whereas Pan et al. (190) did. Our findings align with Pan et al. (190) as significant improvements were observed over time, though no differences were noted between therapies. Regarding SIS, only Chen et al.'s study (188) incorporates this outcome measure, demonstrating significant improvements for the group receiving TMS stimulation. However, this trial revealed a positive trend in both therapies concerning this scale, without significant differences between them or over time.

Lastly, neither of the interventions appeared to alter participants' brain processing speed, as measured by the Finger Tapping Test (FTT) on the unaffected side. This suggests a potential ceiling effect for FTT on the unaffected hemisphere and implies that bilateral rTMS did not disrupt the function of the non-affected hemisphere in terms of FTT and possibly cognitive processing speed.

Nevertheless, it's essential to acknowledge some limitations of this clinical trial. Firstly, it's important to note that the study was not conducted using a randomized double-blind approach. While our results are undoubtedly significant and valuable, we should exercise caution when generalizing these findings to broader contexts. Additionally, there was a difference in the duration of the rTMS and NFB neuromodulation protocols (10 sessions in 2 weeks and 12 sessions in 4 weeks, respectively). This discrepancy, stemming from the design based on previous neuromodulation studies (121,180), could potentially have impacted the results. The assessments were conducted immediately after the conclusion of the entire intervention, which might have missed midterm changes for the group receiving therapy A or immediate effects from rTMS in the therapy B group. Finally, participants continued with their regular neurorehabilitation routine during the treatment, which was not controlled in this research. However, it's important to note that these routines remained consistent for each participant throughout the study period, minimizing their potential impact on the study of treatment effects due to the crossover design.

3.4. OBJECTIVE 4: TO ANALYZE THE CHANGES ON CORTICAL NEUROPHYSIOLOGICAL MEASURES DURING THE EXECUTION OF NFB AND IN RESPONSE TO THE APPLICATION OF THE DESIGNED PROTOCOL FOR POST-STROKE UL REHABILITATION USING A COMBINATION OF NON-INVASIVE BRAIN STIMULATION TECHNIQUES IN LATE SUBACUTE AND CHRONIC PATIENTS.

The use of neurophysiological measures in stroke rehabilitation has emerged as a promising approach to understanding and enhancing the recovery process. These measures, which include electroencephalography and motor evoked potentials and cortical excitability measures, provide insights into the underlying neural mechanisms associated with stroke recovery. These thechniques allow to monitor changes in brain function and activity over time and in response to rehabilitation interventions. Importantly, these neurophysiological measures have been found to correlate with clinical outcomes, providing a potential means of predicting individual responses to treatment and tailoring rehabilitation strategies accordingly (249). This integration of neurophysiological measures into stroke rehabilitation represents a significant advancement in the field, paving the way for more personalized and effective treatment approaches.

Resting Motor Threshold (RMT), Active Motor Threshold (AMT), and Cortical Silent Period (CSP) are neurophysiological measures that can change significantly in stroke patients and in response to rehabilitation.

RMT is an objective measure of cortical excitability, after a stroke, the RMT of the affected hemisphere often increases, indicating decreased cortical excitability. However, with effective rehabilitation, the RMT can decrease again, reflecting improved motor function.

AMT, like RMT, is a measure of cortical excitability but is assessed during slight voluntary muscle contraction. Similar to RMT, AMT of the affected hemisphere often increases after a stroke and can be lowered in response to effective rehabilitation.

CSP is a measure of intracortical inhibitory processes in the motor cortex and is often prolonged in stroke patients, indicating increased inhibition. However, with effective rehabilitation, the CSP can shorten again.

These cortical excitability measures are commonly used to assess the effectiveness of non-invasive neuromodulation protocols using rTMS (Repetitive Transcranial Magnetic Stimulation) and NFB (Neurofeedback) (108,109,111,118,121–124,126).

EEG (Electroencephalography) is a widely used neurophysiological measure as it allows real-time observation of the brain's electrical activity but also allows studies correlating the signals of different locations related to connectivity among different areas. Brain connectivity, measured through EEG, can change because of network adaptations produced by stroke and can also change in response to non-invasive neuromodulation or in correlation with functional recovery after rehabilitation (250).

Different types of EEG recordings may be used to study brain connectivity, the most commonly used is the resting state but the recording during motor paradigms such as finger tapping has also been used.

Interhemispheric symmetry refers to the balance of activity between the two hemispheres of the brain. This balance can be disrupted in the event of a stroke, leading to an imbalance in neural activity between the two hemispheres. The theory of interhemispheric rivalry suggests that after a stroke, the non-lesioned hemisphere could exert an inhibitory influence on the affected hemisphere. This imbalance could impair the recovery of functions (251).

In the context of stroke recovery, the interhemispheric rivalry theory has been used to guide therapeutic interventions. For example, in our case this is the basis for the bilateral rTMS protocol used after the protocol of Takeuchi et al. (121), with the aim of reducing the inhibitory influence on the lesioned hemisphere and stimulating the injured cortex. This approach is based on the idea that by rebalancing the activity between the two hemispheres, it may be possible to enhance the recovery process, so it is likely that changes in EEG signal symmetry and interhemispheric connectivity occur related to our protocol.

As part of this study, we aimed the evaluation of these changes through calculated properties' asymmetry (relative entropy, total power, and waiting time) and the Brain Symmetry Index (BSI), which is a widely used measure in EEG studies.

Entropy is a novel measure that can reveal brain connectivity because as quantifies uncertainty in EEG and the energy or information contained in the signal of different cortical areas, roughly translating to the possible configurations or predictability. Changes in EEG signal complexity and entropy have been correlated with alterations in functional EEG connectivity (252).

Entropy and waiting time properties are derived from physics and have recently been applied to the study of biological signals since they are temporal series with stochastic characteristics. Relative Entropy (253), quantifies how much information a signal contains: less entropy means more information. Waiting time(254), a property similar to activity volatility, is used to detect rhythmic patterns or biological clocks; lower values indicate rhythmic and orderly activity, while higher values suggest disorder. Finally, Total Power is a more common measure, representing the sum of power in different frequency bands (alpha, beta, delta, theta, sigma, and gamma) over a period.

Finally, as part of the complex signal analysis performed during the international stay in LASEEB research group in Lisbon, Portugal, an analysis of event-related potentials is presented, in this case, the EEG signals recorded during the Neurow system training were used. The goal of this complementary analysis was to assess the evolution of these signals from motor areas during training and to identify differential characteristics that may reveal the effect of rTMS on NFB performance.

The validity of neurophysiological measures of cortical excitability and connectivity can constitute prognostic efficacy markers if they are shown to be correlated to clinical variables assessing upper limb functional recovery, the Fugl-Meyer Assessment for the Upper Limb is the gold standard and the main clinical outcome of our trial so this is examined.

3.4.1. CORTICAL EXCITABILITY

3.4.1.1. METHODS

- Patients

20 participants successfully completed the study and were subsequently analyzed. Baseline characteristics of the participants are summarized in Table 7 and demonstrated no statistically significant differences between groups (P>0.05).

Table7. Demographic and clinical characteristics at baseline.

Characteristics	AB Group (N=10)	BA Group (N=10)	P-value
Age, years, mean (SD)	68.50 (9.02)	62.30 (8.32)	0.128
Sex, n (%)			0.371
Female	6 (60%)	4 (40%)	
Male	4 (40%)	6 (60%)	
Time since stroke, months, mean (SD)	27 (21.01)	27.70 (35.51)	0.958
Stroke type, n (%)			0.606
Ischemic	7 (70%)	8 (80%)	
Hemorrhagic	3 (30%)	2 (20%)	
Affected structure, n (%)			0.541
Cortical	6 (60%)	7 (70%)	
Subcortical	3 (30%)	3 (30%)	
Dominance, n (%)			0.305
Right	10 (100%)	9 (90%)	
Left	0 (0%)	1 (10%)	
Affected hemisphere, n (%)			0.178
Right	3 (30%)	6 (60%)	
Left	7 (70%)	4 (40%)	
MoCA, mean (SD)	24.16 (5.08)	23.40 (3.97)	0.115
FMA-UL, mean (SD)	24.50 (16.21)	24.50 (11.84)	0.513

Abbreviations: MoCA: Montreal Cognitive Assessment; FMA-UL: Fugl Meyer Assessment-Upper Limb and SD: Standard Deviation

- Data acquisition and análisis

Subjects are evaluated at six distinct moments, with three evaluations conducted for each therapy. These evaluations encompass an initial assessment conducted before the commencement of the intervention, a subsequent evaluation taking place one week after the intervention's conclusion, and a follow-up assessment two weeks later.

a) *Motor Threshold*

We used a refrigerated figure-of-8 70mm coil connected to a Magstim-Rapid 2 magnetic stimulator (Whitland, UK). The site of stimulation was located at the hotspot based on the motor-evoked potential (MEP) response on the hand corresponding to the stimulated hemisphere using 9 mm diameter Ag-AgCl surface electrodes placed on the first dorsal interosseus (FDI) muscle. EMG measurements were amplified 1000 times, filtered with a band pass of 20 Hz – 2.5 kHz using a Digitimer D440-2 amplifier (Digitimer Ltd., UK) and digitized with a CED Micro 1401-3 (Cambridge Electronic Design, UK). All EMG data were pre-processed and analyzed using Signal software, version 6 (Cambridge Electronic Design, UK) (76)

Subthreshold stimulation intensity was used starting with 40% output intensity single-pulses and gradually increased until motor potentials were evoked.

RMT was defined as the minimal stimulus intensity required to evoke MEPs of at least 50µV in 5 of 10 consecutive trials (240).

AMT was defined as the lowest stimulus intensity to elicit a MEP ≥ 200µV in 5 out of 10 consecutive trials during an isometric contraction of ~10–20% maximal voluntary contraction of the target muscle in the hand.

b) Cortical Silent Period (CSP)

To measure the CSP, during tonic voluntary activation of the target muscle, stimulation intensity is fixed on 120% of the previously calculated RMT. Since the CSP can last up to 200–300 ms, the time window of EMG recording is adjusted to cover at least 400 ms after the TMS stimulus. 10 consecutive trials are administered, and the signal is registered using the previously described Signal software.

In a single trial, the CSP is measured as the time elapsing from the onset of the MEP until the recurrence of voluntary tonic EMG activity. the occurrence of the first visible EMG activity marks the end of the CSP. The final CSP length is result of the average of all the trials.

3.4.1.2. RESULTS

The results for each outcome measure and measurement time are displayed in Table 9. This study investigated three indices of motor cortex excitability in the unaffected hemisphere. As most patients did not exhibit motor evoked potentials when stimulating the affected hemisphere with the maximum intensity recommended by safety parameters (92), we exclusively recorded measurements from the unaffected hemisphere. Table 2 presents the statistical outcomes derived from the mixed linear model, applying a similar analytical approach to that used for the remaining variables.

Figures 23 and 25 show the evolution of the average AMT and RMT respectively in each measure. Figure 26. Shows the evolution of CSP.

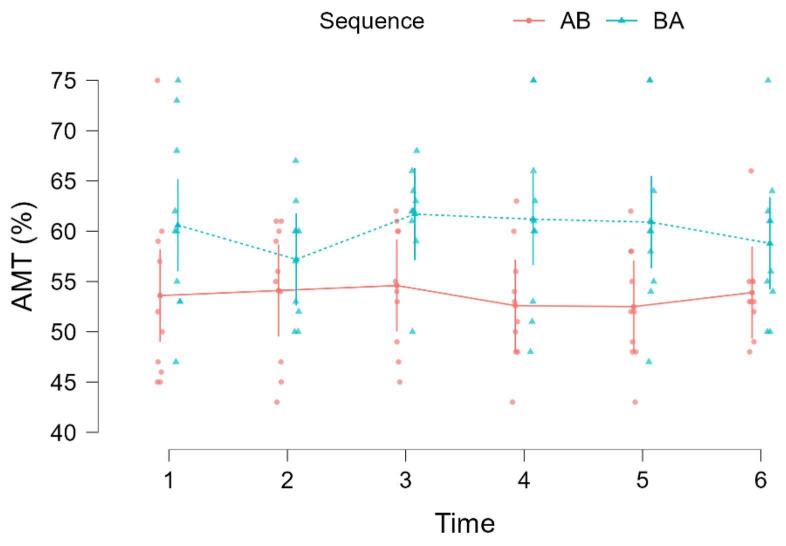

Figure 24. Changes in Action Motor Threshold over time, according to the treatment sequence.

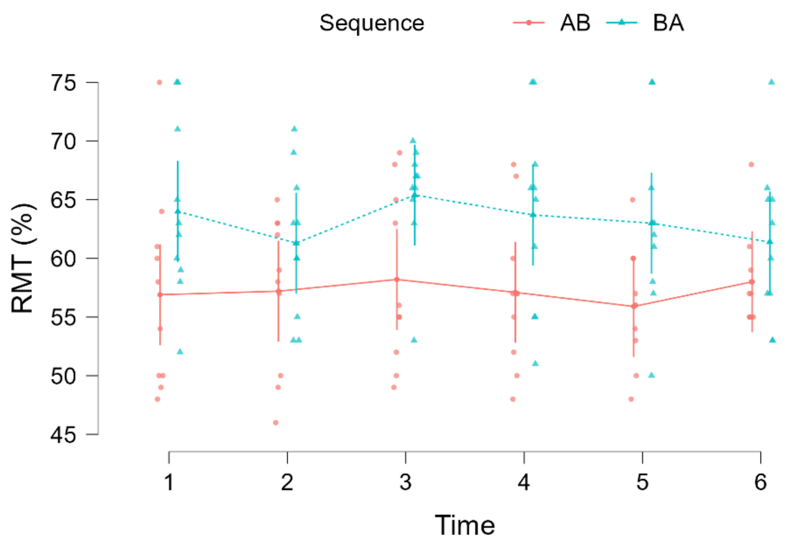

Figure 25. Changes in Resting Motor Threshold over time, according to the treatment sequence.

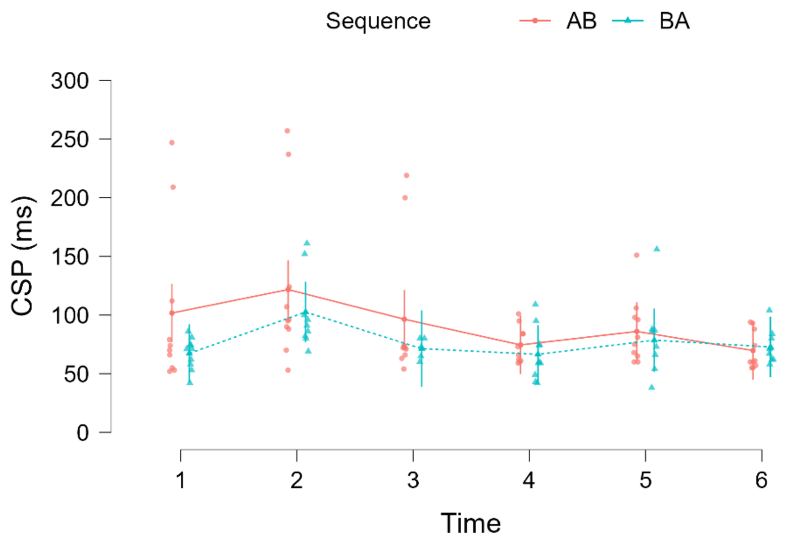

Figure 26. Changes in Cortical Silent Period over time, according to the treatment sequence.

3.4.2. ELECTRICAL BRAIN SYMMETRY AND CONNECTIVITY

3.4.2.1. METHODS

- Patients

Out of the 20 participants who completed the study, 18 individuals were included in the subsequent analysis. Ten subjects were part of the AB treatment sequence, while eight were in the BA sequence. The baseline characteristics analysis indicated no statistically significant differences between the groups (P>0.05).

- Data acquisition and analysis

Subjects are evaluated at six distinct points, with three evaluations conducted for each therapy. These evaluations encompass an initial assessment conducted before the commencement of the intervention, a subsequent evaluation taking place one week after the intervention's conclusion, and a follow-up assessment two weeks later.

- EEG acquisition.

An actiCHamp amplifier (Brain Vision LLC, NC, USA) was used to amplify and digitize the EEG data at a sampling frequency of 512 Hz. The EEG data were stored in a PC running Windows 7 (Microsoft Corporation, Washington, USA). EEG activity was recorded from 64 positions with active Ag/AgCl scalp electrodes (actiCAP electrodes, Brain Vision LLC, NC, USA). The ground and reference electrodes were placed on AFz and on FCz, respectively (see Figure 27).

EEG acquisition was carried out by NeuroRT Studio software (Mensia Technologies SA, Paris, France).

Resting EEG activity was recorded over two minutes. The patients were comfortably seated with their hands on their laps, relaxed jaw and eyes open, looking at a white wall. Immediately afterwards, they were instructed to tap their index finger against the thumb for 30 s with the maximum amplitude and speed possible. The healthy hand was the first and then the other, if movements could not be elicited the patient was asked to do as bes as he can or imagine the movement.

- Electroencephalographic asymmetry properties

- Signal Total Power: It is defined as the accumulated quadratic intensity over a measurement, for a specific frequency band and electrode. This value represents a measure of the total energy devoted to the production of a given signal and can be calculated in both the time and frequency domains using the following relationships:

$$Total\ Power = \sum |s_i(t)|^2 = \sum |\tilde{s}_i(\omega)|^2$$

Here, $\tilde{s}(\omega)$ denotes the Fourier Transform of the electroencephalographic signal.

- Spectral Relative Entropy: It serves as an indicator of homogeneity in the energy distribution across different frequencies within a signal. It can be calculated using the equation:

$$H_{rel} = (-\sum P(\omega_i) \log P(\omega_i)) / H_{max}$$

Here, $P(\omega i)$ represents the power fraction corresponding to frequency ωi in the overall signal. Simultaneously, H_{max} denotes the maximum entropy value, which, under specific conditions, corresponds to the entropy of white noise, where all frequencies are equally represented. Thus, relative entropy is confined to the interval [0-1]. Values close to unity indicate an even energy distribution among different frequencies, whereas smaller values suggest power concentration within a narrow frequency range.

- Waiting Time Variance: It is defined as the normalized variance of the residence times of the signal in quantized states, identified as regions of values above or below the signal's mean voltage. To perform this calculation, initially, the signal is quantized using the operation:

$$s_{c,i}(t) = 1/2\ (1 + Sign\,[s_i(t)])$$

Once the signal is binarized, the residence times of the signal in each of the states are calculated. From these, the variance of the residence time can be defined as:

$$WTV = Var\,(t)\ /\ \langle t \rangle^2$$

The dynamic interpretation of these residence times is related to the variability in the stochastic oscillations of electroencephalographic signals. Small values indicate that the waves exhibit well-defined repetitive oscillatory patterns, known as biological clocks, while large values indicate that the signal demonstrates strong stochastic behavior.

It's worth noting that the process of signal binarization may obscure rapid oscillations if they have low power. Therefore, the use of this property is particularly suitable for band analysis rather than the analysis of the signal across the entire frequency spectrum.

-Brain Symmetry Index (BSI): It is defined as the relative difference in powers, for a specific band, between hemispheres:

$$BSI = 1/K \sum |\ (Rn - Ln)/(Rn + Ln)\ |$$

Where $Rn = 1/C \sum_i |\tilde{s}_i(\omega)|^2$ represents the mean power of the electrodes. Normalization of this property confines it to the interval [0,1], such that values close to zero indicate equivalent power between both hemispheres for a specific band. Values approaching one suggest a substantial difference in power between hemispheres for a given band.

a) *Signal pre-processing*

The electroencephalogram (EEG) captures signals from 64 electrodes distributed across different areas of the scalp. These analog signals are susceptible to environmental noise and random elements due to the conditions under which they are collected. Therefore, preprocessing is necessary to 'clean' the signal for accurate subsequent analysis.

To perform preprocessing, the EEGLAB tool (255), implemented in MATLAB, was utilized. The preprocessing steps undertaken using this program, in sequence:

1. *Elimination of frequencies higher than 100Hz:* Using a band-pass filter, signal components with frequencies exceeding 100 Hz were removed, effectively

eliminating significant noise, primarily induced by external environmental elements or electrode placement defects.

2. *Removal of noise and artifacts* using the Artifact Subspace Reconstruction (ASR) method, with a standard parameter set to 20 (rejecting data if they exceed 20 times the standard deviation of signal components).
3. *Channel (electrode) analysis* to identify channels without a signal and potential reconstruction based on neighboring signals if necessary.
4. *Signal digitization for subsequent property extraction using software*. After digitizing the signal, two actions were taken to standardize the analysis:
   - To ensure comparability among extracted properties, all signals were trimmed to a total time of 30 seconds.
   - Focusing solely on analyzing the patient's motor response, only channels (electrodes) corresponding to signals produced by the brain's motor areas were utilized (Figure 27).

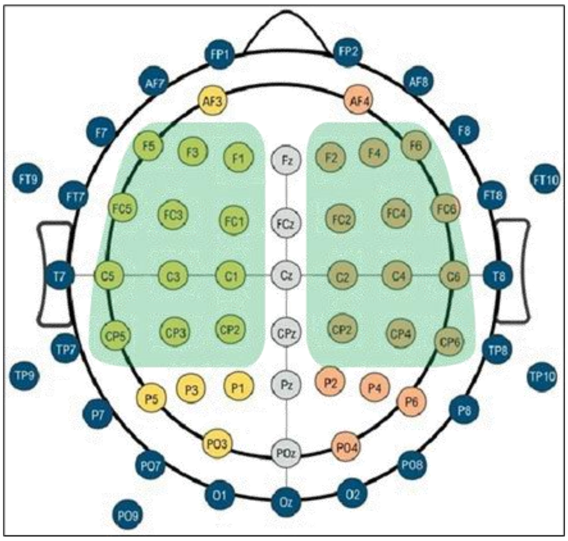

Figure 27. Electrodes selected for capturing electrical brain signals.

b) Signal Referencing and signal filtering

Once artifacts have been removed, the next step involves signal referencing, if applicable. In this study, a reference-free system (Raw references) has been chosen. This system merely involves rescaling each signal where the electrode's voltage is rescaled concerning the mean value in the observation window:

$$S_{RAW}(t) = S(t) - \langle S\ (t) \rangle_i$$

This procedure maintains the dynamic components of stochastic signals. However, the signals exhibit high levels of contamination between electrodes.

Following the previous step, the referenced signals undergo subsequent bandpass filtering using a Finite Impulse Response (FIR) filter with a Hamming window, selecting the total frequency range (1-30 Hz).

c) Signal processing

Once we have selected the referencing type (RAW) and frequency band (TOTAL), dynamic properties were calculated: Signal total power, spectral relative entropy, and waiting time variance.

To establish the asymmetry between brain hemispheres based on these properties, the following estimator was computed, confined to the motor regions of both hemispheres, Standardized Mean Difference (SMD):

$$SMD = |\langle x_i \rangle_L - \langle x_i \rangle_L|^2 / 0.5\ (\sigma\ (x_L) + \sigma\ (x_R))$$

Where $\langle x_i \rangle_L$ and $\langle x_i \rangle_R$ represent the mean values of the selected property for the left and right hemispheres, respectively, and $\sigma(x_L)$ and $\sigma(x_R)$ the standard deviations, respectively.

3.4.2.2. RESULTS

The results for total power, relative entropy, waiting time and BSI for all measurements and pre post changes in resting state and finger tapping are shown in table 11. Regarding asymmetry, each outcome measure and measurement time point are presented in tables 13 and 14. As stated in the methods section, the study examined the interhemispheric asymmetry of three dynamic properties and one standardized symmetry measurement during three patient states: resting state with eyes closed, right finger tapping task, and left finger tapping task. In table 12, the statistical results obtained using the mixed linear model can be observed, following the same analysis as with the rest of the variables.

- Total Power

Overall, no significant differences were found between therapies, the sequence of therapy administration or interactions with time in asymmetry according to the total power property during rest. Significant differences were found in the interaction between sequence and time during FTT in the affected hemisphere (Figure 28-29).

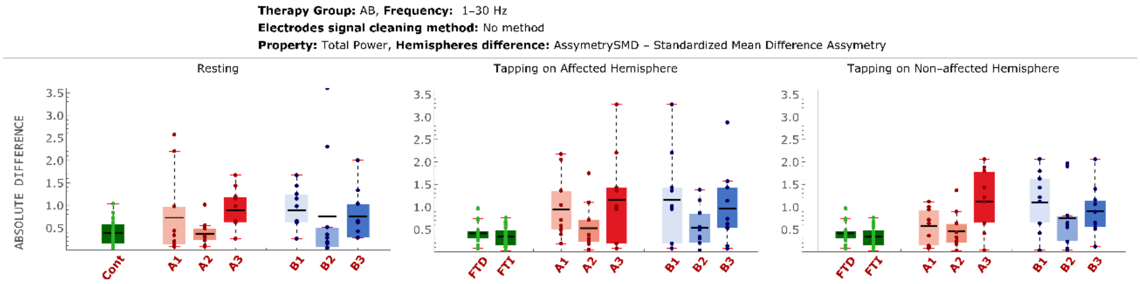

Figure 28. AssymetrySMD. total power AB.

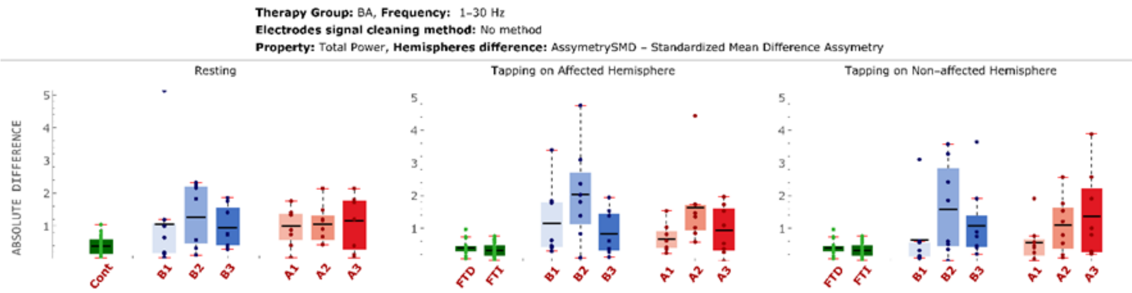

Figure 29. AssymetrySMD. total power BA.

- <u>Relative Entropy</u>

In general, no statistically significant variances were observed in terms of therapy comparisons, therapy sequence variations, or time-related interactions regarding asymmetry as assessed through the total power property during the resting state. Nevertheless, notable distinctions were identified in the interaction involving sequence and time in the context of FTT in the affected hemisphere. (Figure 30-31).

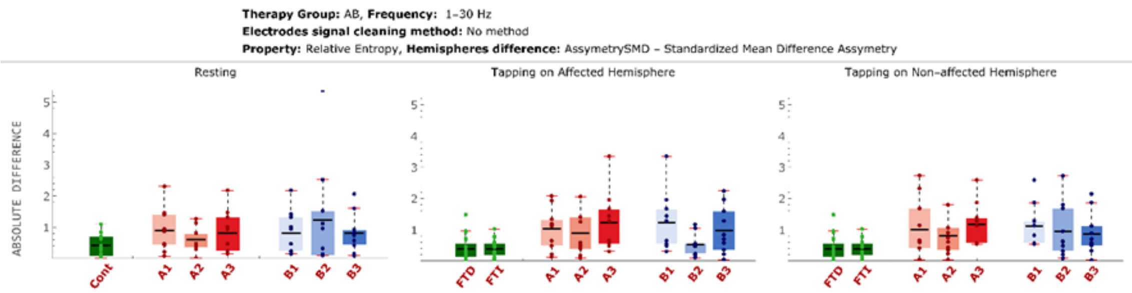

Figure 30. AssymetrySMD. relative entropy AB.

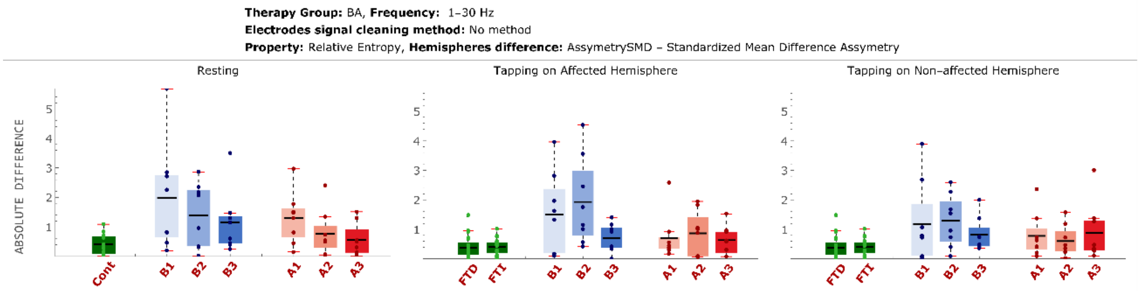

Figure31. AssymetrySMD. relative entropy BA.

- <u>Waiting Time</u>

Regarding waiting time, there were no significant effects of therapy, the sequence of therapy administration, or interactions with time were found in asymmetry during FTT. However, significant differences were observed in asymmetry during resting state over time in relation to this property. (Figure 32-33)

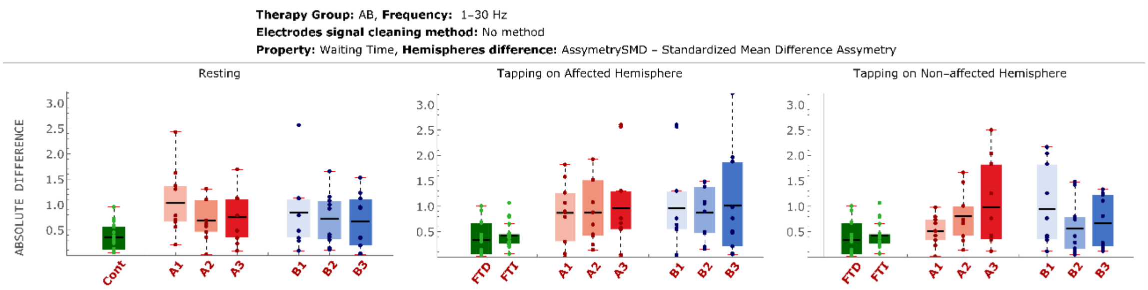

Figure 32. AssymetrySMD. waiting time AB.

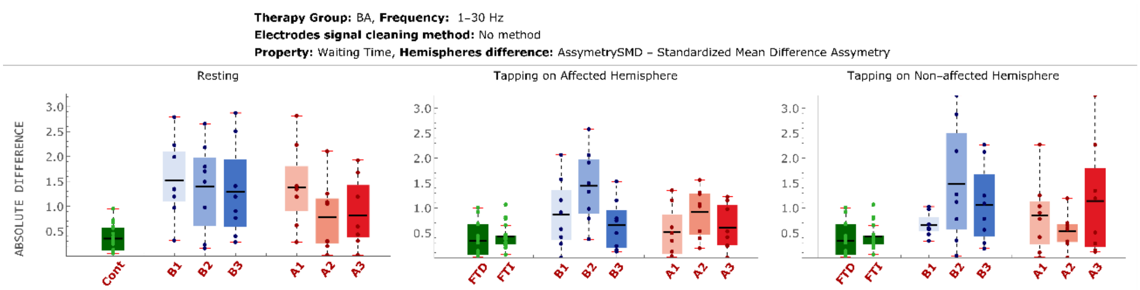

Figure 33. AssymetrySMD. waiting time BA.

These results suggest that asymmetry in different EEG properties responds differently to therapies and the sequence of therapies in patients with stroke.

However, it's essential to consider that further research and analysis are needed to fully understand the clinical implications and applicability of these findings in the evaluation of NIBA effects on stroke patients.

- Brain symmetry Index

Overall, there were no statistically significant differences found when comparing therapies, variations in therapy sequence, or interactions related to time concerning asymmetry, as evaluated through the BSI during FTT. However, significant differences were noted specifically in the administration sequence of the therapies concerning resting state. (Figure 34-35).

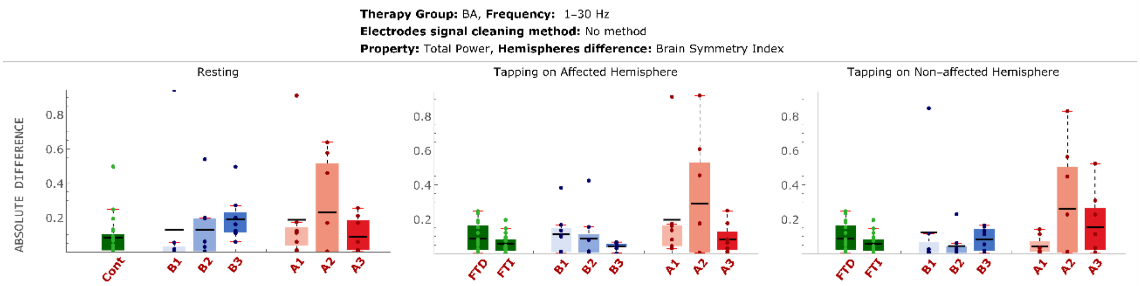

Figure 34. Brain Symmetry Index. AB

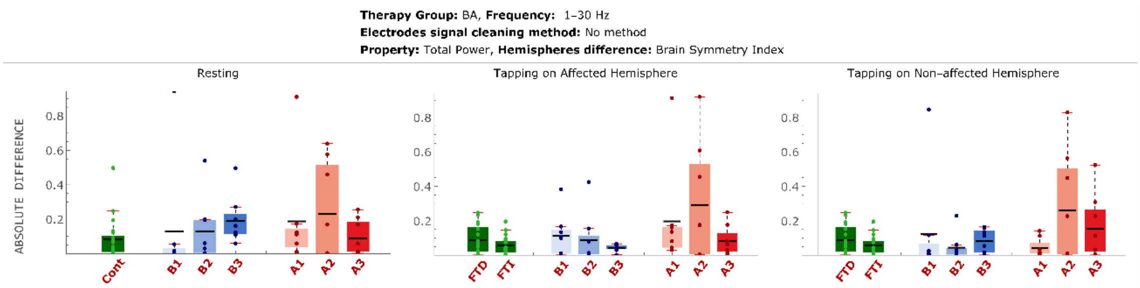

Figure 35. Brain Symmetry Index. BA

It is noteworthy that during the resting state, the BSI value decreases following both therapies in patients who undergo the BA therapy sequence. However, those who followed the AB sequence showed a decrease in BSI after therapy A, followed by an increase after therapy B. In relation to this value, and in line with published studies (256), repetitive bilateral transcranial magnetic stimulation appears to be effective in reducing cerebral asymmetry at rest following a stroke.

Regarding changes in BSI during the FT, we encounter the opposite scenario. Patients who followed the BA sequence exhibited a decrease in BSI after therapy B, and an increase after therapy A. Concerning the AB sequence, both therapies result in a decrease in BSI. Based on the analysis of BSI during a motor task, it appears that MI-based NFB following repetitive transcranial magnetic stimulation is effective in reducing cerebral asymmetry after a stroke.

3.4.3. EVOLUTION OF EEG EVENT-RELATED POTENTIALS DURING MI-BASED NFB TRAINING

3.4.3.1.METHODS

- Patients

A total of 10 participants from those that successfully completed the study were subsequently analyzed. Only patients who had initiated the BA sequence were selected to eliminate any potential carryover effects from prior bilateral rTMS stimulation.

The baseline characteristics of the participants are summarized in Table 8.

Table 8. Demographic and clinical characteristics at baseline.

Characteristics	Patients (N=10)
Age, years, mean (SD)	62.30 (8.32)
Sex, n (%)	
Female	4 (40%)
Male	6 (60%)
Time since stroke, months, mean (SD)	27.70 (35.51)
Stroke type, n (%)	
Ischemic	8 (80%)
Hemorrhagic	2 (20%)
Affected structure, n (%)	
Cortical	7 (70%)
Subcortical	3 (30%)
Dominance, n (%)	
Right	9 (90%)
Left	1 (10%)
Affected hemisphere, n (%)	
Right	6 (60%)
Left	4 (40%)
MoCA, mean (SD)	23.40 (3.97)
FMA-UL, mean (SD)	24.50 (11.84)

Abbreviations: MoCA: Montreal Cognitive Assessment; FMA-UL: Fugl Meyer Assessment-Upper Limb and SD: Standard Deviation

- Data acquisition and analysis

EEG data were acquired using the BCI-connected EEG system provided by Brainproducts, consisting of 64 active electrodes with low-noise biosignal amplifiers and a 24-bit A/D converter operating at 256 Hz.

Electrodes were spatially organized in relevant areas, including frontal (F3, Fz, F4), frontal central (FC5, FC6), central (C3, Cz, C4), central parietal (CP5, CP1, CP2, CP6), and parietal (P3, Pz, P4), following the 10-20 EEG configuration. EEG data acquisition and processing were conducted via the OpenVibe platform, utilizing the Lab Streaming Layer (LSL) protocol to interface with the virtual environment.

The initial phase of data acquisition involved collecting raw EEG data for training a classifier to distinguish between imagined left- and right-hand movements using the NeuRow VR-BCI paradigm (180). The training sessions included 24 randomized blocks per class (left or right hand). Data underwent spatial and temporal filtering to create feature vectors. Spatial filtering aimed to remove noise from the EEG signals, while temporal filtering focused on the alpha and beta frequency bands (8 to 30 Hz). The classifier was then trained with this preprocessed dataset. Throughout the study, only the raw EEG data from the training sessions were analyzed, although online data was also available.

Patients adhered to a comprehensive protocol spanning a 4-week duration. During the initial two weeks, patients received 10 sessions of bilateral rTMS concurrently with 6 sessions of MI-based NFB employing NeuRow system training. In the subsequent two weeks, participants exclusively underwent 6 sessions of MI-based NFB therapy. Bilateral rTMS was administered every weekday, while MI-based NFB therapy sessions were conducted three times per week. A visual representation of the protocol timeline is provided in Figure 36.

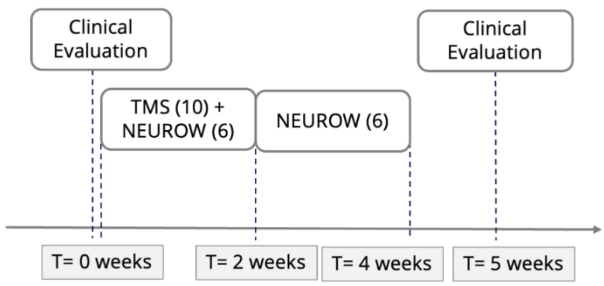

Figure 36. Experimental protocol timeline.

a) *Pre-processing Pipeline Design*

The pre-processing pipeline design is a crucial aspect of this study. The data from the 12 MI-based NFB sessions conducted over two weeks for each of the 10 patients were used. However, ten sessions had to be discarded due to various issues like patient discomfort, incorrect EEG configurations, or inaccurate data format. Since the data were collected from real patients in a less controlled environment than a laboratory, it was susceptible to artifacts, which could be of technical or biological origin (257).

Technical artifacts arise from equipment issues, while biological artifacts stem from patient physiology or behavior. In this case, pre-processing was more extensive than usual, which could potentially remove important data (258). Two primary methods were used: Artifact Subspace Reconstruction (ASR) (259) and Independent Component Analysis (ICA) to address this (260). ASR identifies high-variance signal fragments exceeding a given threshold and removes them, while ICA separates independent components of the EEG signal. The choice between ASR and ICA depended on the aggressiveness needed for each session. It was observed that the ASR algorithm was more efficient in terms of time but removed more signal, whereas ICA was less aggressive but preserved more data. Ultimately, the ASR algorithm was chosen for its noise-reduction capabilities. Variable pre-processing was also considered, applying more aggressive pre-processing to sessions deemed 'bad' based on the number of channels and trials removed. However, it was found that the variable approach was not viable for noisy signals. Thus, the final pre-processing pipeline was designed to remove as much noise as possible to address environmental and patient-related noise issues.

b) *Preprocessing Pipeline*

The EEG signal underwent a comprehensive preprocessing pipeline using the EEGLAB (255) toolbox in MATLAB. The pipeline involved several steps (Figure 37).

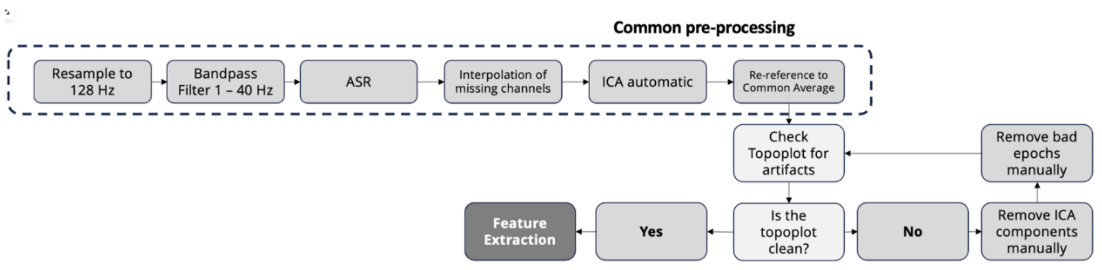

Figure 37. Schematic of the overall final pre-processing pipeline.

c) *Event-Related Desynchronization*

In the context of this work, Event-Related Desynchronization (ERD) represents a decrease in the power of the alpha band because of movement preparation or motor imagery. The formula for computing ERD is as follows (180):

$$ERS/ERD = (PMI - PBL) / PBL * 100\%$$

Here, ERS and ERD stand for Event-Related Synchronization and Desynchronization, respectively. PMI and PBL represent the average power of a certain frequency band (such as alpha) for the motor imagery section of the EEG and the baseline of the EEG, respectively. The baseline refers to the 3 seconds prior to a trigger event in the EEG when the subject is in a resting state.

To calculate the ERD value, the time-frequency matrix was obtained using the newtimef function, which computes the power of a signal using the Fast Fourier Transform method (FFT) (261) . A Hann window was applied to the signal before FFT to reduce leakage effects. The time-frequency matrix represents the average matrix across all trials.

To compute ERD for a given session, the steps involved included averaging the values in the alpha band (8-13Hz) across time, obtaining a vector of ERD values per time point, and then averaging from 0.5 seconds since the trigger until the 4-second mark to account for reaction time and prolonged ERD.

The ERD values per time point were computed using a sliding window of length 86, chosen to match the number of samples in the baseline. Sliding window analysis was employed due to the noisy nature of ERD, caused by the hospital setting and the cortical damage in the patients. The ERD was extracted from electrodes C3 and C4, corresponding to the brain areas more active during arm movements, with C3 and C4 representing the contralateral side of the movement for left and right epochs, respectively.

This approach allowed the characterization of ERD patterns during motor imagery for each patient and session (Figure 38).

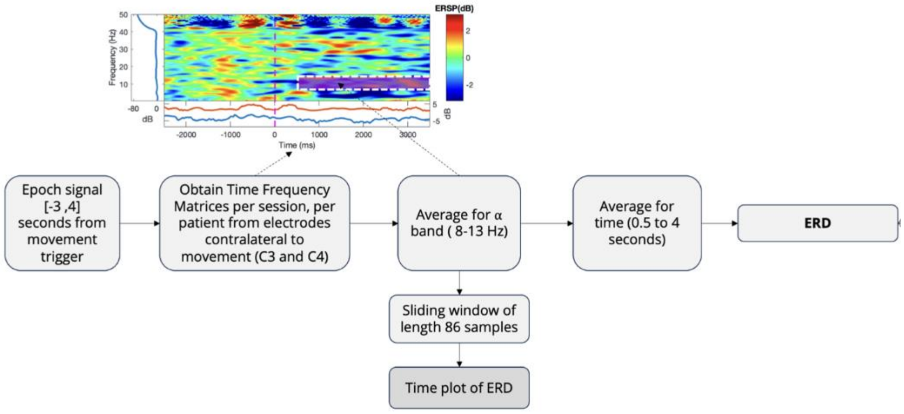

Figure 38. The process involves averaging the alpha band from the Event-Related Spectral Perturbation matrix to obtain ERD values for each patient and session.

3.4.3.2. RESULTS

In Figure 39, there is no discernible pattern in how patients' Event-Related Desynchronization (ERD) changes across different sessions, as ERD values show fluctuations during sessions. However, except for sessions 9 and 12, nearly all sessions show statistically significant differences when compared to zero. Detailed p-values for these comparisons and median ERD values per session can be found in Table 15.

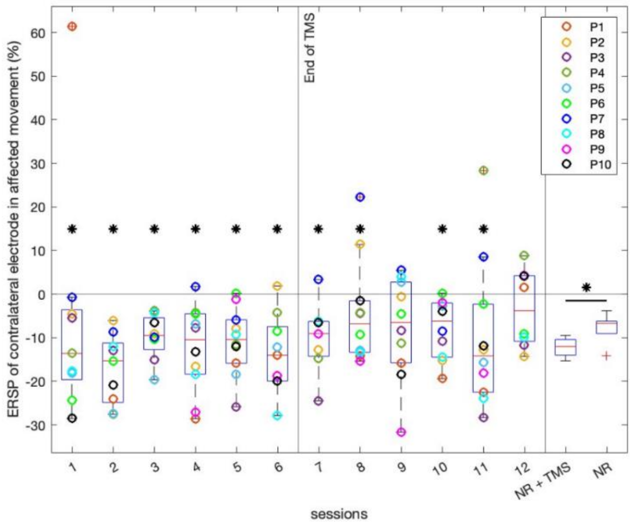

Figure 39. ERD values for the AH per patient per session.

Furthermore, patients who consistently exhibit more positive or more negative ERD values maintain this characteristic across sessions. For example, Patient 7 (depicted in dark blue) consistently has ERD values that are less negative than the median. In contrast, Patient 1 exhibits an extreme outlier in session 1, but generally, their ERD values are more negative than the median. Notable extreme outliers also include patients 7 and 4 in sessions 8 and 11, respectively.

Table 15 shows that the ERD medians during the period when both therapy technologies were administered are more negative than when only BCI was used. To assess the significance of this difference, the distributions of medians (Med = -12.0307% for the rTMS protocol and Med = -6.6666% for the non-rTMS protocol) were compared, and they were found to be statistically different with a p-value of 0.04112 (Figure 39).

To provide further insights into ERD patterns when all 10 patients undergo rTMS sessions, the time profile of alpha frequency band power was extracted from the last session of rTMS and MI-based NFB training and compared to the last session of MI-based NFB training alone. The results are depicted in Figure 40, representing the brain area contralateral to the paralyzed limb. The blue line in this plot represents the average ERD responses of all patients, while the grey shaded area corresponds to the standard deviation among all patients. These plots were generated by averaging the time-frequency matrix for the alpha band from all trials in a single session for each patient and then averaging all resulting time variations of Event-Related Spectral Perturbation (ERSP) in a specific session for all patients.

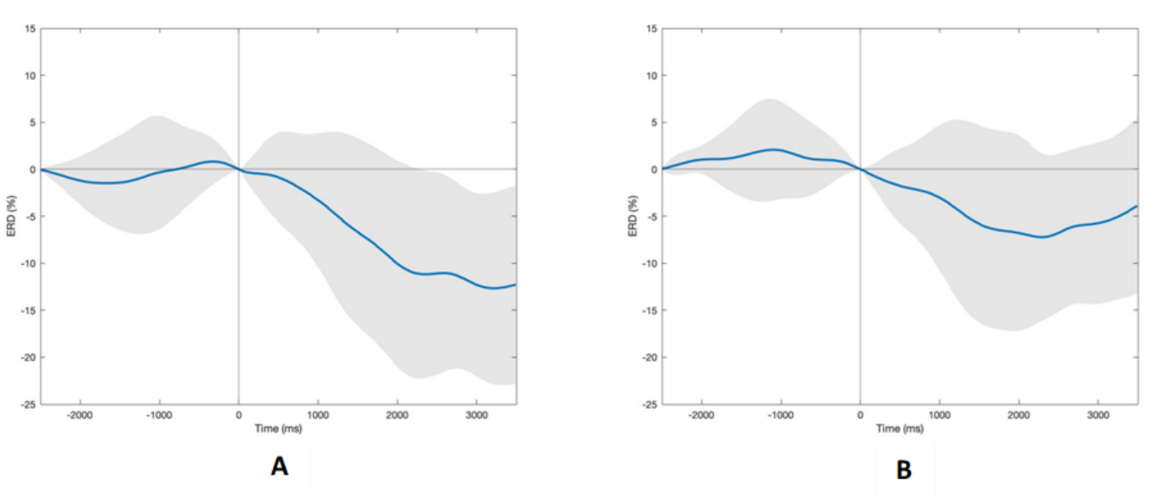

Figure 40. Time profile of the alpha frequency band power for contralateral electrode to the AH.

From Figure 40, it can be inferred that ERD time patterns are similar to those found in the literature (262). Furthermore, when patients undergo both therapies, the blue line exhibits a more negative slope (slope = -5.427 $\times 10^{-3}$ % / ms) compared to when only NFB training is applied (slope = -2.6029 $\times 10^{-3}$ % / ms). Additionally, ERSP is sustained for a longer duration (t = 1171.84 ms) when patients receive both therapies compared to when only NFB training is applied (t = 749.98 ms). Please note that these sessions were selected to illustrate the periods with and without rTMS, rather than focusing on their evolution.

3.4.4. CLINICAL VARIABLES CORRELATION WITH NEUROPHYSIOLOGICAL MEASURES

First, we calculated the pre-post changes in the neurophysiological variables and FMA-UL. Then, we assessed the normality of these changes using the Shapiro-Wilk statistical test, given the relatively small sample size. Since the Shapiro-Wilk p-value yielded values greater than 0.05, we assumed that these changes follow a normal distribution. Assuming this normality, we computed correlations using the Pearson statistical parameter (Table 16).

No significant correlations were found between changes in neurophysiological variables and changes in FMA-UL ($p > 0.05$).

Table 9. Measurement of cortical excitability parameters.

Variable	TMS Measurements					
	1	**2**	**3**	**4**	**5**	**6**
RMT. mean (SD)						
AB	56.90 (8.50)	57.20 (0.66)	58.20 (7.44)	57.10 (6.57)	55.90 (5.02)	58.00 (4.06)
BA	64.00 (7.59)	61.30 (6.34)	65.40 (4.79)	63.70 (8.21)	63.00 (7.69)	61.40 (6.80)
AMT. mean (SD)						
AB	53.60 (9.45)	54.10 (6.79)	54.60 (6.13)	52.60 (5.97)	52.50 (5.78)	53.90 (4.89)
BA	60.60 (9.13)	57.20 (5.79)	61.70 (4.83)	61.20 (9.16)	60.90 (8.77)	58.80 (7.53)
CSP. mean (SD)						
AB	101.70 (69.36)	121.70 (68.90)	96.40 (60.08)	74.50 (15.52)	86.00 (28.15)	69.70 (16.14)
BA	67.30 (13.45)	101.89 (32.35)	71.60 (8.96)	66.50 (22.01)	81.00 (35.06)	73.33 (14.31)

Abbreviations: AMT: Action Motor Threshold; CSP: Cortical Silent Period; RMT: Resting Motor Threshold; SD: standard desviation.

Table 10. ANOVA mixed model summary.

Variable	Effect	df	F	P-value
Action Motor Threshold	Time	5,90	0.52	>0.05
	Sequence	1,18	7.03	0.016*
	Time-sequence	5,90	0.87	>0.05
	Therapy	1,18	0.54	>0.05
Resting Motor Threshold	Time	5,90	0.74	>0.05
	Sequence	1,18	6.66	0.019*
	Time-sequence	5,90	0.61	>0.05
	Therapy	1,18	0.55	>0.05
Cortical Silent Period	Time	5,82	4.36	0.001*
	Sequence	1,19	1.56	>0.05
	Time-sequence	5,82	0.86	>0.05
	Therapy	1,19	4.03	0.049*

Table 11. Measurement of EEG asymmetry properties (Asymmetry SMD mean and standard deviation (SD)).

Variable	Measurements of electroencephalographic asymmetry properties					
	1	2	3	4	5	6
Resting State. mean (SD)						
Total Power						
AB	0.72 (0.92)	0.37 (0.27)	0.89 (0.48)	0.89 (0.45)	0.75 (1.21)	0.75 (0.56)
BA	1.05 (0.62)	1.26 (0.62)	0.94 (0.85)	0.98 (1.50)	1.05 (0.92)	1.14 (0.64)
Relative Entropy						
AB	0.90 (0.68)	0.60 (0.39)	0.82 (0.69)	0.81 (0.68)	1.23 (1.66)	0.82 (0.61)
BA	1.97 (0.83)	1.38 (0.72)	1.14 (0.56)	1.30 (1.66)	0.77 (0.99)	0.55 (0.99)
Waiting time						
AB	1.03 (0.65)	0.68 (0.39)	0.75 (0.51)	0.84 (0.71)	0.71 (0.51)	0.67 (0.55)
BA	1.52 (0.78)	1.39 (0.67)	1.29 (0.69)	1.38 (0.86)	0.78 (0.74)	0.82 (0.88)
Brain Symmetry Index (BSI)						
AB	0.09 (0.12)	0.04 (0.04)	0.07 (0.09)	0.07 (0.10)	0.03 (0.03)	0.07 (0.06)
BA	0.13 (0.32)	0.12 (0.19)	0.19 (0.14)	0.19 (0.30)	0.23 (0.28)	0.09 (0.10)
FTT (Affected Hemisphere). mean (SD)						
Total Power						
AB	0.95 (0.70)	0.53 (0.53)	1.16 (0.99)	1.17 (0.99)	0.53 (0.46)	0.96 (0.84)
BA	1.15 (0.60)	2.04 (0.53)	0.82 (0.69)	0.68 (0.43)	1.64 (1.20)	0.93 (0.74)
Relative Entropy						
AB	1.03 (0.67)	0.90 (0.74)	1.22 (0.94)	1.22 (0.94)	0.52 (0.35)	0.98 (0.76)
BA	1.32 (1.43)	1.63 (1.60)	0.67 (0.52)	0.71 (0.79)	0.87 (0.76)	0.63 (0.49)
Waiting time						
AB	0.87 (0.61)	0.88 (0.61)	0.96 (0.93)	0.96 (0.93)	0.87 (0.51)	1.01 (1.03)
BA	0.87 (0.70)	1.44 (0.73)	0.65 (0.50)	0.52 (0.50)	0.92 (0.49)	0.60 (0.46)
Brain Symmetry Index (BSI)						
AB	0.13 (0.13)	0.07 (0.12)	0.09 (0.10)	0.09 (0.09)	0.01 (0.02)	0.06 (0.06)
BA	0.11 (0.13)	0.08 (0.15)	0.04 (0.02)	0.19 (0.29)	0.29 (0.34)	0.08 (0.09)
FTT (Non-Affected Hemisphere). mean (SD)						
Total Power						

AB	0.58 (0.39)	0.46 (0.40)	1.12 (0.68)	1.10 (0.65)	0.74 (0.68)	0.90 (0.55)
BA	0.64 (1.02)	1.57 (1.40)	1.08 (1.16)	0.56 (0.60)	1.09 (0.85)	1.36 (1.32)
Relative Entropy						
AB	1.00 (0.94)	0.80 (0.61)	1.18 (0.67)	1.12 (0.68)	0.95 (0.85)	0.87 (0.67)
BA	1.16 (1.40)	1.29 (0.89)	0.82 (0.58)	0.79 (0.75)	0.60 (0.53)	0.89 (0.98)
Waiting time						
AB	0.51 (0.29)	0.81 (0.49)	0.98 (0.87)	0.94 (0.81)	0.56 (0.54)	0.67 (0.52)
BA	0.65 (0.24)	1.48 (1.17)	1.07 (0.78)	0.84 (0.72)	0.54 (0.35)	1.14 (1.13)
Brain Symmetry Index (BSI)						
AB	0.09 (0.09)	0.07 (0.06)	0.07 (0.05)	0.07 (0.05)	0.06 (0.12)	0.09 (0.07)
BA	0.12 (0.29)	0.04 (0.08)	0.08 (0.07)	0.04 (0.05)	0.26 (0.32)	0.15 (0.19)

Abbreviations: AH: affected hemisphere; FTT: Finger Tapping Task; nAH: non affected hemisphere; SD: standard deviation.

Table12. ANOVA mixed model summary.

Variable	Effect	df	F	P-value		Effect	df	F	P-value
Resting_totalpower	Time	5,88	0.59	>0.05					
	Sequence	1,17	2.77	>0.05					
	Time-sequence	5,88	0.19	>0.05					
	Therapy	1,28	0.75	>0.05					
FTT_AH_totalpower	Time	5,8	0.7	>0.05	FTT_nAH_totalpower	Time	5,8	1.87	>0.05
	Sequence	1,16	3.45	>0.05		Sequence	1,16	1.12	>0.05
	Time-sequence	5,8	3.54	0.006		Time-Sequence	5,8	1.58	>0.05
	Therapy	1,11	1.1	>0.05		Therapy	1,11	0.58	>0.05
Resting_Rel_entropy	Time	5,88	1.76	>0.05					
	Sequence	1,17	1.24	>0.05					
	Time-sequence	5,88	0.99	>0.05					
	Therapy	1,49	2.95	>0.05					
FTT_AH_rel_entropy	Time	5,8	0.86	>0.05	FTT_nAH_rel_entropy	Time	5,8	0.87	>0.05
	Sequence	1,16	0.06	>0.05		Sequence	1,16	0.11	>0.05
	Time-Sequence	5,8	2.71	0.02		Time-Sequence	5,8	0.38	>0.05
	Therapy	1,22	1.62	>0.05		Therapy	1,22	1.29	>0.05
Resting_waitingtime	Time*	5,88	2.27	0.05					
	Sequence	1,17	1.43	>0.05					
	Time-sequence	5,88	1.32	>0.05					

	Therapy	1,71	2.06	>0.05					
FTT_AH_waitingtime	Time	5,8	0.92	>0.05	FTT_nAH_waitingtime	Time	5,8	1.08	>0.05
	Sequence	1,16	0.37	>0.05		Sequence	1,16	1.42	>0.05
	Time-Sequence	5,8	1.24	>0.05		Time-Sequence	5,8	2.08	>0.05
	Therapy	1,47	3.34	>0.05		Therapy	1,47	1.22	>0.05
Resting_BSI	Time	5,80	0.54	>0.05					
	Sequence	1,16	5.90	0.03					
	Time-Sequence	5,80	0.53	>0.05					
	Therapy	1,67	0.01	>0.05					
FTT_AH_BSI	Time	5,96	1.49	>0.05	FTT_nAH_BSI	Time	5,96	1.27	>0.05
	Sequence	1,96	2.97	>0.05		Sequence	1,96	2.26	>0.05
	Time-Sequence	5,96	0.38	>0.05		Time-Sequence	5,96	1.74	>0.05
	Therapy	1,104	0.39	>0.05		Therapy	1,104	1.513	>0.05

Abbreviations: AH: affected hemisphere; BSI: Brain Symmetry Index; FTT: Finger Tapping Task; nAH: non affected hemisphere.

Table13. Measurement of EEG entropy by hemisphere in resting (mean).

		RESTING							
		THERAPY A				THERAPY B			
		1	**2**	**3**	**Δ1-2**	**1**	**2**	**3**	**Δ1-2**
Total Power	**AH**	11534320,7	11747410210	872000	3609122769	24920083	13341,163	46238775	-24906742,5
	nAH	10537897,1	3619660666	872460,37	11735875889	29317280,	983008,53	519175,3	-28334272,3
Relative Entropy	**AH**	0,6309	0,6569	0,6678	0,026	0,6703	0,667	0,6626	-0,0033
	nAH	0,6272	0,6612	0,667	0,034	0,6703	0,6689	0,6557	-0,0014
Waiting Time	**AH**	0,18453009	0,227489701	0,0212061	0,042959612	0,0197148	0,0439963	0,009387	0,02428156
	nAH	0,11185813	0,209750066	0,0259319	0,097891938	0,0195733	0,0400705	0,104944	0,02049713

Abbreviations: AH: affected hemisphere; nAH: non affected hemisphere.

Table14. Measurement of EEG entropy by hemisphere in motor task (mean).

		FINGER TAPPING							
		THERAPY A				THERAPY B			
		1	**2**	**3**	**Δ1-2**	**1**	**2**	**3**	**Δ1-2**
Total Power	**AH**	0,82798759	1,02395975	1,06300611	0,19597216	1,15998949	1,20346912	0,90226492	0,04347963
	nAH	0,56992926	0,73938928	1,22832404	0,16946002	0,89297817	1,11272405	0,98217726	0,21974587
Relative Entropy	**AH**	0,88864017	0,88686033	0,96057351	-0,00177984	1,35364448	1,14824834	0,85967834	-0,2053961
	nAH	0,90335442	0,70885446	1,05075138	-0,19449996	1,1410263	1,09909979	0,8450259	-0,0419265
Waiting Time	**AH**	0,71018468	0,89827868	0,80049186	0,18809401	0,91997708	1,12239508	0,852749	0,202418
	nAH	0,65862136	0,68867078	1,04860396	0,03004943	0,81292426	0,96671511	0,84695276	0,15379085

Abbreviations: AH: affected hemisphere; nAH: non affected hemisphere.

Table15. Median and p-value of ERD when compared to 0 for sessions with Therapy B.

	Therapy B											
	Bilateral rTMS and MI-based NFB training						**MI-based NFB training**					
Sessions	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
ERD Median (%)	-13.59	-15.33	-9.50	-10.46	-10.42	-14.01	-9.04	-6.83	-6.50	-6.23	-14.19	-3.81
p-value	0.001	<0.001	<0.001	0.001	0.001	0.001	0.006	0.017	> 0.05	0.001	0.017	> 0.05

Abbreviations: ERD: Event Related Desynchronization; MI: Motor Imagery; NFB: Neurofeedback and rTMS: Repetitive Transcranial Magnetic Stimulation.

Table16. Neurophysiological and FMA-UL changes correlations.

VARIABLE	PEARSON'S CORRELATIONS	
	Pearson's r	**p-value**
ΔRMT-ΔFMA-UL	-0.861	0.139
ΔAMT-ΔFMA-UL	-0.895	0.105
ΔCSP-ΔFMA-UL	0.836	0.164
ΔResting_Total_Power-ΔFMA-UL	0.690	0.310
ΔResting_Relative_Entropy-ΔFMA-UL	-0.113	0.887
ΔResting_Waiting_Time-ΔFMA-UL	-0.830	0.170
ΔFTT_AH_Total_Power-ΔFMA-UL	0.225	0.775
ΔFTT_AH_Relative_Entropy-ΔFMA-UL	0.319	0.681
ΔFTT_AH_Waiting_Time-ΔFMA-UL	0.454	0.546
ΔFTT_nAH_Total_Power-ΔFMA-UL	0.495	0.505
ΔFTT_nAH_Relative_Entropy-ΔFMA-UL	0.742	0.258
ΔFTT_nAH_Waiting_Time-ΔFMA-UL	0.773	0.227
ΔResting_BSI-ΔFMA-UL	0.715	0.246
ΔFTT_AH_BSI-ΔFMA-UL	0.651	0.364
ΔFTT_nAH_BSI-ΔFMA-UL	0.634	0.383

Abbreviations: AH: affected hemisphere; AMT: Action Motor Threshold; BSI: Brain Symmetry Index; CSP: Cortical Silent Period; FMA-UL: Fugl Meyer Assessment-Upper Limb; nAH: non affected hemisphere; RMT: Resting Motor Threshold; FTT: Finger Tapping Task.

3.4.5. DISCUSSION

According to the interhemispheric inhibition theory, the unaffected hemisphere exercises an inhibitory activity over the affected hemisphere jeopardizing its recovery. Our protocol intended to inhibit the unimpaired hemisphere reducing its excitability. Nevertheless, we observe that after therapy B, regardless of the sequence of administration, the resting and action motor thresholds tended to decrease, implying an increase in the excitability of the unaffected side. In the case of therapy A, the trend varied according to the treatment sequence. In the AB sequence, there was an increase in thresholds, and in the BA sequence, there was a decrease in thresholds, both of which are very minimally significant.

When statistical studies were performed, there were no significant differences in AMT or RMT concerning time which suggests that despite the described trends, these measures remain relatively stable during the assessment period throughout the therapies. These measurements do not detect a clear change in the excitability of the motor cortex in the unaffected hemisphere in response to the applied therapies.

Nevertheless, the significant interaction between the sequence of therapy administration and both AMT and RMT suggests that the order in which therapies are administered can impact the excitability of the motor cortex in the unaffected hemisphere, which may be result of the carry over effect evidenced in FMA assessment.

Our findings are consistent with the majority of prior studies, indicating that contralateral cortical excitability, as assessed by motor thresholds, does not exhibit significant differences compared to the findings in the existing literature (121,122,126) However, it's worth noting that our findings differ from two previous studies (108,124), which do report significant increase after inhibitory rTMS stimulation, possibly due to the use of unilateral application, among other factors. These disparities could stem from variations in patient populations, methodologies employed, or diversity in therapeutic protocols.

On the other hand, when CSP changes were evaluated, the presence of significant differences in unaffected hemisphere CSP concerning time suggests that this measure changes in response to the therapies. The increase of the lenght of this period on unaffected hemisphere reveals an increase of intracortical inhibition, allegedly through potentiation of GABAergic mechanisms (263). Most of the clinical symptoms observed after stroke are not solely due to the lesion itself but also atributed to the hyperactivity recorded in the intact hemisphere, which indirectly inhibits the affected one (264). Our finding of increased CSP duration is clearly result of the efficacy of the inhibitory rTMS stimulation applied on the unaffected hemisphere.

The lack of significant differences concerning the sequence of therapy administration indicates that CSP response may not depend on the order in which therapies are administered and in this case is not sensitive to the carry over effect evidenced in FMA.

Moreover, the significant differences when comparing this change between therapies shows a bigger increase when therapy B is applied (rTMS plus MI-based NFB), this means that although NFB has a global activity and is not intended to specifically inhibit the unaffected hemisphere, it potentiates the effect of rTMS. As shown in this thesis, therapy B also shows significant greater improvement compared to therapy A in functional arm performance measured through FMA although, as shown, its correlation with CSP changes is not statistically significant.

Regarding CSP, it's interesting to note that only two previous studies measured this variable (108,109), and our comparability aligns more closely with one of them, which also assessed the contralateral hemisphere (108). However, our study reports significant changes after both therapies, in contrast to the lack of significance shown by the changes in the study by Bashir et al. (108), in which unilateral inhibitory rTMS over unaffected hemisphere is applied. This may suggest that CSP is a sensitive measure of therapeutic effects of unaffected hemisphere inhibition, and our alignment with a prior study supports it should be examined as a possible marker for assessing cortical excitability in the context of post-stroke rehabilitation. However, the limited number of studies that have measured CSP underscores the need for future research to confirm and expand upon these findings.

Assessing the brain's electrical responses to specific therapies through EEG can yield valuable insights to evaluate rehabilitation effectiveness (265). EEG responses may vary depending on the paradigm used during the recording (266–268) so is of crucial importance to evaluate different paradigms such as resting state and finger tapping.

In general brain asymmetry in response to non-invasive neuromodulation does not change in a uniform manner in all the studied properties.

After a stroke, an increase in cerebral entropy has been observed in some cases, indicating greater variability in brain activity as part of the recovery response (269). As the brain adapts to compensate for damaged areas, new neural networks are activated, resulting in increased variability in brain activity (entropy). Additionally, functional connectivity changes occur between different brain regions, especially in the early stages of recovery, where connectivity between damaged and healthy areas may decrease due to the injury (270). Over time and during recovery, it is likely that connectivity between these regions is restored and, in some cases, increased, which may be related to the greater variability in brain activity during the restoration of normal brain functions. These changes in entropy and cerebral connectivity are interrelated and may indicate plastic adaptations in the brain during the recovery process following a stroke (271).

The relative entropy calculations by hemisphere are consistent with the findings reported in the existing literature (272), as entropy during a motor action or motor imagery is greater than during resting state (273). Both the affected and unaffected cerebral hemispheres tend to experience a decrease in relative entropy following the neuromodulation with both therapies in finger tapping recordings and increase just in response to therapy B during resting state. This observation may suggest that there is more information on each electrode and thus probably associated with enhanced intrahemispheric connectivity. However, this trend is not uniformly observed during resting state where this reduction only happens after therapy B.

Regarding total power calculation, both hemispheres show an increase after both therapies in finger tapping state and decrease after therapy B during resting state. This observation aligns with similar findings in older adults and stroke patients (274).

Finally, the property called waiting time also increases after both therapies in resting state and in Finger tapping, the interpretation of this variable beyond symmetry is complex as greater values are related with less rhythmic or ordered signals meaning possibly that the intrahemispheric connectivity is increased.

Beyond intrahemispheric connectivity, interhemispheric asymmetry is a crucial variable in post stroke brain function. In some cases, a certain degree of asymmetry may be necessary for recovery, as the unaffected hemisphere can compensate for lost functions in the affected hemisphere (275) reflecting a positive recovery process (249). However, persistent or imbalanced asymmetry can be detrimental if it hinders functional recovery (249). Therefore, the evaluation of cerebral asymmetry is an interesting phenomenon that could give complementary insights to the effects of rehabilitation and neuromodulation therapies.

When analyzing the change trends regarding asymmetry of the calculated variables, it becomes evident that the property showing the most pronounced changes is "Total Power," which exhibits a clear change in measurements following the administration of both therapies. However, the trends vary depending on the therapy sequence. In the AB sequence, "Total Power" values decrease after both therapies, becoming more symmetric, while they increase following the BA sequence, increasing asymmetry.

When examining the variables "Relative Entropy" and "Waiting Time," the changes are less pronounced compared to "Total Power." Similar to the previous property, there are no clear trends, as the observed changes vary depending on the therapy sequence.

The therapies itself (A or B) did not appear to exhibit significant differences concerning any assimetry dynamic properties.

Concerning BSI, in our study with chronic and subacute patients, no statistically significant differences were found when comparing therapies, variations in therapy sequence, or time-related interactions concerning asymmetry measured by BSI during the FTT. However, notable patterns were observed specifically in the therapy administration sequence during the resting state, displaying a different trend between the BA and AB sequences.

These findings align with the article by Zhong et al. (256), which concluded that when both brain hemispheres were simultaneously activated, rTMS decreased interhemispheric asymmetry during resting state. It is worth noting that although the application was bilateral, the stimulation parameters differed, and the population included in that study encompassed patients with unilateral traumatic brain injury. This is the sole study found that analyzes changes in BSI following rTMS. Concerning the relationship between cerebral asymmetry and upper limb motor recovery in stroke patients, our study contrasts with the results of Saes et al. (268) and Sebastian Romagosa et al. (276), who found significant correlations between BSI and FMA-UL. Additionally, Saes et al.'s (266) results in subacute patients during the resting state did not reflect significant changes in BSI after, contrary to our observations in chronic and subacute patients.

These discrepancies among studies may suggest significant variability in the post-stroke cerebral asymmetry dynamics across different clinical stages and conditions. Although our current findings do not reveal significant differences in BSI during the FTT, the patterns identified in the resting state imply the relevance of therapy sequences in modulating cerebral asymmetry. These results highlight the need for future research to better understand how therapeutic sequences influence cerebral asymmetry and its implications in post-stroke rehabilitation among chronic and subacute patients.

Regarding the modulation of Event-Related Desynchronization (ERD) in post-stroke patients through both therapy techniques, the findings revealed that most sessions exhibited significant ERD modulation, indicating that patients could effectively regulate their sensorimotor rhythms during therapy. This capacity for modulation is crucial, as it has been associated with clinical recovery in previous studies (277). Specifically, a decrease in ERD has been linked to clinical improvement. Here, we observe that when rTMS was applied, patients exhibited significantly lower ERD, suggesting a positive effect of rTMS (278).

An intriguing aspect of the results was the observation that, as soon as rTMS was discontinued, ERD values became less negative compared to the initial phase of therapy. This raises questions about the temporality of rTMS recovery and suggests that positive effects may depend on the maintenance of therapy. Therefore, long-term follow-up of these patients would be essential to assess ongoing effects on performance and patient recovery. The lack of a discernible trend in ERD modulation among patients could be attributed to various factors. Firstly, the hospital environment may have introduced noise into the measurements. Additionally, brain lesions and compromised cortical integrity in patients may have weakened the ERD response. Moreover, patient motivation, especially in cases where patients expressed demotivation, could have influenced their responses (279).

One possible reason for the ERD results could be related to the high-frequency stimulation of rTMS, which has been shown to promote the formation of new neural connections. This could explain the decrease in observed alpha power levels. Specifically, it might be due to increased asynchronous neuronal activity during motor imagery or a higher baseline activity in the alpha frequency band, resulting in elevated baseline alpha power.

Finally, the statistical data obtained from the correlation between the neurophysiological variables and FMA-UL are in line with a recently published systematic review (50), where no significant correlations were found.

4. GENERAL DISCUSSION

This thesis aimed to address four significant objectives related to the application of non-invasive neuromodulation techniques as a complement to conventional neurorehabilitation with the objective to potentiate the recovery of upper limb motor performance of patients who have experienced a stroke in subacute or chronic phases. Each of the proposed objectives and their respective results and discussion offer a comprehensive view of the research conducted and its findings.

Objective 1 focused on reviewing the current evidence regarding the effects of rTMS on improving UL motor function in patients in the subacute and chronic stages following a stroke. The results of this systematic review suggest that rTMS offers notable benefits in the rehabilitation of these patients. The review revealed that, regardless of the timing of treatment initiation, bilateral rTMS has a positive impact on UL functionality, as assessed through various functional tests. These findings support and summarize previous studies that reported favourable effects of rTMS in the acute phase of stroke recovery.

While previous research had confirmed the efficacy of rTMS in improving general UL motor function, as measured through the FMA-UL, this study expanded the scope by considering other aspects of motor function, such as strength, dexterity, and muscle tone that were shown to be also improved by rTMS. Additionally, measures of cortical excitability were reported in various studies although, it was found that neurophysiological evolution does not always align with the motor and clinical evolution of the affected upper limb.

Finally, this review highlighted some significant limitations in the current literature. Substantial heterogeneity among studies in terms of sample size, location of brain lesions, and types of strokes poses challenges in generalizing the results. The lack of a clear consensus on the optimal dosing parameters for rTMS in stroke rehabilitation was also emphasized, despite the overall safety of these interventions. Further research with more homogeneous outcome measures and intervention protocols are needed to perform meta-analysis that yield evidence-based recommendations that allow the recommendation of concise rTMS protocols in stroke rehabilitation.

Objective 2 involved designing a clinical trial protocol to investigate the clinical impact of MI-based NFB training after bilateral rTMS in individuals who have experienced a stroke with more than three months of post-stroke evolution. This approach represents an innovation in neurorehabilitation by exploring the synergy of rTMS and MI-based NFB.

The combination of rTMS and NFB is based on the idea that these two complementary interventions may have an additive effect on motor function recovery as rTMS. Previous evidence has indicated differences in the scope of neuromodulatory effects between endogenous modulation through MI-based NFB and exogenous modulation via rTMS, with MI-based NFB potentially showing a wider influence on subcortical regions, while rTMS tends to exert its primary impact on cortical areas (280).

Objective 3 was directed to finally examine the clinical effects induced by the proposed protocol in late subacute and chronic stroke patients.

Nevertheless, the protocol underwent a significant change in terms of its design. Originally, the experimental design was planned as parallel, randomized, controlled, consisting of two groups of 21 subjects each one. The first group receiving bilateral rTMS application preceding the MI-based NFB training and the second one just bilateral rTMS.

However, it became evident that due to economic limitations it was impossible to complete the recruitment of all the sample. To be able to compare both interventions and considering the available resources, a sample of 20 subjects was predefined and shifting to a crossover design with a washout period allowed us to extract preliminary conclusions to examine the feasibility of a future paralel trial according to the calculated sample. In this new design, all the recruited patients received both therapies, serving as both active subjects and controls. Several modifications were implemented concerning the primary outcome variables. Given our utilization of a gold standard scale for assessing upper limb function, we reclassified AMI as a secondary variable. The array of primary variables was extensive, and our focus was directed towards investigating the clinical effectiveness of the employed techniques across three specifically identified areas, which were extensively deliberated. These areas encompass changes related to rTMS, MI, NFB, and VR: UL functionality, isometric grip strength, and sensory assessment. These adjustments were motivated by the pursuit of a more comprehensive functional assessment that would enhance sensitivity in detecting potential outcomes. This refinement was particularly crucial as the original sample was not being utilized.

The execution the clinical trial, adapted as described, examined the clinical effects of combining MI-based NFB training with bilateral repetitive transcranial magnetic stimulation for UL motor function in subacute and chronic stroke patients. The results indicated that this combined approach led to significant improvements in UL motor function, particularly as measured by the FMA-UL, meaning that functional improvements were evident. However, hand-grip strength did not exhibit significant differences between the two therapeutic strategies, somatosensory sensation did improve. Our results must be considered as preliminary due to the limited sample included and the modified design, although the evidenced trends suggest that this combination may enhance UL recovery after a stroke.

However, it is also important to acknowledge that this study was not conducted using a randomized double-blind approach, raising the possibility of biases. Additionally, differences in the duration of rTMS and NFB protocols may have influenced the results. Finally, the inclusion of standardized conventional rehabilitation therapy as part of participants' treatment routines during the study should also be considered in further trials proving this protocol.

Finally objective 4 was an ambitius attempt to look for potential neurophysiological markers of non-invasive neuromodulation efficacy or prognostic markers. The studies were focused on examining changes in brain excitability, cortical inhibition, cerebral connectivity, and sensorimotor rhythms through various neurophysiological measures. The findings indicated that although motor thresholds showed minor changes, they did not significantly reflect alterations in motor cortex excitability due to the therapies. This may be in part justified by the difficulty of the consistent register of these variables due to their known variability. However, changes in the cortical silent period (CSP) suggested increased intracortical inhibition following inhibitory rTMS, particularly on the unaffected hemisphere as was intended using the bilateral protocol inhibiting the healthy hemisphere. These changes were indicative of potential therapeutic effects over the cortical neurophysiological variables but weren't significantly influenced by the sequence of therapy administration which had differential effects when the clinical variables were examined.

Interestingly, the functional improvement measured by the Functional Motor Assessment (FMA), wasn't correlated to any neurophysiological variables.

As a second part of this objective, we explored cerebral asymmetry dynamics and found variable trends in brain asymmetry-related measures such as total power, relative entropy, and waiting time, with varying responses to the different therapies and their sequence with no clear conclusions of these changes that on the other hand, did not either correlated with clinical variables.

The lack of correlation between neurophysiological variables and functional motor assessment was consistent with prior research, showing no significant correlations, underscoring the complexity of these relationships in post-stroke rehabilitation.

In the last part of this objective, we studied the modulation of event-related desynchronization (ERD), which is known to be crucial for sensorimotor function. The sessions exhibited significant modulation during therapy, particularly showcasing decreased ERD levels with rTMS, indicating its positive effect on potentiating sensorimotor cortex function. However, the sustainability of this effect after therapy cessation remains uncertain, highlighting the need for long-term patient follow-up.

In conclusion, the results of the systematic review and the results of the preliminary clinical trial suggest that rTMS but mainly its combination with MI-based NFB, have the potential to improve UL motor function and somatosensory sensation in subacute and chronic stroke patients. These findings are promising for the development of more effective and personalized rehabilitation strategies in the future. However, further research is needed to refine treatment protocols and address the limitations identified in this study.

5. CONCLUSIONS

1. The systematic review of evidence supports the application of bilateral repetitive transcranial magnetic stimulation (rTMS) as an effective intervention to enhance upper limb motor function in patients in the subacute and chronic stages after a stroke. (H1)

2. The systematic review of evidence demonstrated that significant improvements in various aspects of motor function, including strength, dexterity, and muscle tone may be produced by bilateral rTMS protocols. (H1)

3. The proposed clinical trial protocol aims to assess the clinical efficacy of motor imagery-based neurofeedback (MI-NFB) training following bilateral repetitive transcranial magnetic stimulation (rTMS) in patients who have experienced a stroke with more than three months of post-stroke evolution. (H2)

4. The combination of rTMS and MI-NFB is considered a multidimensional strategy in neurorehabilitation with the potential to improve motor and somatosensory function in chronic stroke patients. (H2)

5. The combination of rTMS and MI-NFB resulted in significant enhancements in upper limb motor function, particularly as assessed by the Fugl-Meyer Assessment for Upper Limb (FMA-UL). (H3)

6. Hand-grip strength did not exhibit significant differences between the two therapeutic strategies, suggesting their equal effectiveness in this regard. (H3)

7. Improvements in somatosensory sensitivity, especially in touch and movement tests, favours the combined therapy. (H3)

8. Significant changes in intracortical inhibition were identified in healthy hemisphere, potentially reflecting the effects of inhibitory rTMS contributing to reducing post-stroke inhibitory hyperactivity over the injured hemisphere. (H4)

9. While no significant differences were detected in cerebral symmetry during motor tasks, notable patterns emerged in cerebral asymmetry at rest, suggesting the potential relevance of therapeutic sequencing in this modulation. (H4)

10. The effects of rTMS in modulating Event-Related Desynchronization (ERD), may indicate enhanced patient capacity to effectively regulate sensorimotor rhythms when the combined neuromodulation protocol is applied. (H4)

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7. APPENDICES

7.1. PROMOTIONAL PROCHURE FOR THE *HANDBOOST* PROJECT

7.2. PROMOTIONAL VIDEO FOR THE *HANDBOOST* PROJECT

7.3. TESTIMONIALS FROM PARTICIPANTS IN THE *HANDBOOST* PROJEC

7.4. INFORMED CONSENT

Título: **Estudio HandboosTMS. Uso de estimulación magnética transcraneal repetitiva como terapia coadyuvante en la rehabilitación de miembro superior en ictus cerebral**

CONSENTIMIENTO INFORMADO PARA LA ESTIMULACIÓN MAGNETICA TRANSCRANEAL

Descripción del estudio:

Ha sido invitado a participar en una investigación centrada en la potenciación de la rehabilitación del miembro superior mediante el uso de la técnica de estimulación magnética transcraneal (TMS). La TMS es un procedimiento no invasivo que utiliza un campo magnético que es capaz de modificar levemente la actividad del cerebro durante un tiempo limitado (entre segundos y minutos).

Podrá abandonar el experimento en el momento que lo desee. Por favor, lea cuidadosamente este documento y pregunte a los investigadores o al personal del estudio cualquier palabra o información que no entienda con claridad.

SEGURIDAD DEL ESTUDIO

Los parámetros de la TMS utilizados en este estudio son propios de la investigación llevada a cabo con dicha técnica, y cumplen todos los criterios de seguridad establecidos por consenso en el año 2009 por el "Safety of TMS Consensus Group" y otros investigadores de prestigio internacional (Rossi et al., 2009).

Por lo que se refiere a los potenciales beneficios de esta investigación, es necesario tener presente que la realización de esta técnica ha demostrado en varios estudios previos producir una ligera a moderada mejoría en la rehabilitación del miembro superior. Sin embargo se ha visto que esta mejoría en caso de producirse es temporal durando de horas a días, por este motivo se le propondrá una serie de sesiones repetidas con la intención de prolongar el mayor tiempo posible los efectos motores y cognitivos del procedimiento.

Si se da el caso de que no experimente la mejoría esperada (aunque pueda ser muy sutil y solo evidente para sus médicos), los datos obtenidos pueden facilitar el conocimiento de las bases neurales de estos trastornos y abre las puertas al uso potencial de nuevas herramientas terapéuticas.

PROCEDIMIENTO TMS

Usted permanecerá sentado en una silla mientras el investigador, o alguien de su equipo, coloca la bobina de estimulación sobre su cabeza, apoyándola de forma cómoda para usted sobre su cuero cabelludo. La bobina produce un campo magnético que puede modificar brevemente la actividad de su cerebro. Cada vez que se genera un pulso magnético se oirá un *click*, y puede sentir una breve contracción en el músculo del cuero cabelludo. Lo sentirá como si fuera un golpecito o un pellizco en el cuero cabelludo.

Para encontrar la intensidad de la estimulación magnética necesaria para usted, los investigadores primero medirán los pulsos de TMS sobre una parte de su cerebro que controla el movimiento de los dedos. Su movimiento muscular será registrado por unos electrodos colocados en su mano. El investigador comprobará cual es la intensidad mínima de la estimulación magnética necesaria para crear un pequeño movimiento muscular en su dedo. Este número es diferente en cada persona y servirá para fijar la intensidad de estimulación de la TMS que se empleará durante el estudio.

Esta parte del estudio se llevará a cabo en una única sesión experimental de aproximadamente 50 minutos de duración.

Posteriormente como parte del protocolo de rehabilitación se realizarán sesiones de estimulación magnética transcraneal repetitiva en las cuales se administra una gran cantidad de pulsos magnéticos de baja intensidad en una zona determinada del cerebro (en su caso la zona que mueve la mano), este procedimiento se hace bilateralmente al igual que el procedimiento descrito anteriormente y su duración es de aproximadamente 30 minutos. Estas sesiones se repetirán diariamente (en días laborables) por 10 días.

PROCEDIMIENTO NEUROW

Usted permanecerá sentado en una silla con los electrodos del EEG colocados sobre su cuero cabelludo. Usted deberá realizar imaginería mental de la mano correspondiente, en base a los estímulos presentados en la pantalla del ordenador. En primer lugar, se llevará a cabo una sesión de entrenamiento para que se familiarice con el "neurojuego".

Durante las sesiones, los pacientes verán un bote y dos brazos virtuales agarrando dos remos, vista en primera persona. Deberán imaginar el movimiento de cada mano correspondiente para rotar cada remo y progresar, observando el movimiento imaginado en pantalla. La interfaz del juego incluye cronometraje y puntuación. El objetivo de la tarea es recopilar tantas banderas como sea posible en un período de tiempo fijo. Se registrará el número de banderas recolectadas en cada sesión. Se realizarán en 12 sesiones no consecutivas (3 sesiones a la semana) de 30 minutos cada una, divididas en 3 series para prevenir la fatiga.

EVALUACIÓN COGNITIVA

Antes y después de terminar el ciclo de modulación con TMS se le administrará un breve examen del estado mental, un cuestionario de efectos secundarios y un cuestionario sobre su estado emocional. En algunos casos, se le podrán aplicar antes de las sesiones con TMS algunos test neuropsicológicos y para evaluar su función motora para evaluar la evolución de los síntomas que se estudian en la investigación.

POSIBLES RIESGOS, EFECTOS SECUNDARIOS Y MALESTARES

La TMS ha sido utilizada en investigación desde hace más de 20 años, y se han desarrollado guías de seguridad. Hay diferentes tipos de TMS y en este estudio los investigadores seguirán todas las recomendaciones de seguridad para el tipo de TMS que se utilizará. Aún siguiendo las recomendaciones de seguridad pueden aparecer los siguientes efectos secundarios.

<u>*Efectos secundarios más comunes*</u>

- Dolor de cabeza y cuello: puede sufrir dolor de cabeza o de cuello después de la TMS. Se debe a la tensión muscular por mantener erguida de la cabeza y el cuello durante la TMS. Entre el 20 y el 40% de los sujetos que se han sometido a TMS sufre dolor de cabeza. Si le aparece dolor de cabeza le ofreceremos paracetamol o aspirina que le aliviará del dolor de cabeza producido por la TMS. En algunos casos, la TMS causa malestar facial momentáneo en el lado de la cara en el que se ha administrado la TMS. La molestia cede al suspender el procedimiento.

- Molestias auditivas: el chasquido que produce la TMS puede provocar un zumbido leve en los oídos o cambios temporales en su capacidad para oír los sonidos de frecuencias más bajas. El uso de tapones o auriculares puede prevenir de forma efectiva la presencia de molestias auditivas posteriores, por lo que se le proporcionaran tapones durante el experimento. Si durante el experimento su tapón se afloja o se cae avise al investigador. No podrá participar en el estudio si tiene historial de problemas auditivos severos que hayan requerido implante coclear.

Efectos secundarios poco frecuentes

- Convulsiones: la TMS puede causar convulsiones en muy raras ocasiones. Si se cumplen los parámetros de seguridad es un hecho altamente improbable. Los investigadores utilizarán las preguntas del cribado para reducir el riesgo de que ningún participante tenga historia de riesgo de sufrir convulsiones.
- Experimentar convulsiones causadas por la TMS no quiere decir que vaya a sufrir más convulsiones. No significa que tenga epilepsia. No significa que deba tomar medicación para prevenir futuras convulsiones. Los sujetos que han tenido convulsiones debidas a la TMS no tienen porqué continuar teniendo problemas de salud relacionados con las convulsiones.

- Síncope (desvanecimiento): es posible que pueda desvanecerse durante la TMS. Es muy infrecuente, pero puede suceder si está ansioso, nervioso o si no ha comido. Deberá informar inmediatamente al equipo si siente vértigo, mareo o siente que va a perder el conocimiento. La TMS se interrumpirá y será monitorizado hasta que se sienta mejor.

- Memoria: la TMS puede ocasionar cambios en la memoria, la atención y otras funciones cognitivas. Sin embargo, no se ha documentado que ninguno de estos efectos sea duradero, son muy leves y son extremadamente poco frecuentes.

Aunque la TMS ha sido utilizada mundialmente desde 1984, puede haber complicaciones que todavía no se conozcan.

DERECHOS DE LOS INVESTIGADORES DEL ESTUDIO

Los investigadores tienen el derecho de interrumpir su participación en el estudio si determinan que no adecuado que continúe en él, si pudiera ser peligroso para usted continuar o si no sigue los procedimientos del estudio como le indican los investigadores.

Autorización

Por este documento solicitamos su autorización para realizarle el procedimiento, así como a usar imágenes e información de su Historia Clínica con fines docentes o científicos. Su anonimato será respetado.

Declaración y firmas

Antes de firmar este documento, si desea más información o tiene cualquier cuestión, no dude en preguntarnos.

Información básica relativa a la protección de sus datos de carácter personal:

El responsable del tratamiento de sus datos es la Universidad Francisco de Vitoria (UFV).

Conforme a lo dispuesto en la LOPD (Ley de Protección de Datos) Reglamento (UE) 2016/679 del Parlamento europeo y del Consejo de 27 de abril de 2016 de Protección de Datos (RGPD). Se informa de que sus datos serán tratados e incorporados al fichero de la FUNDACIÓN UNIVERSIDAD FRANCISCO DE VITORIA con fines de gestión, investigación científica y docencia. Sólo podrían ser cedidos a organismos autorizados. Puede ejercitar sus derechos de acceso, rectificación, supresión, oposición, limitación del tratamiento y portabilidad mediante un escrito dirigido a la Secretaría General de la Universidad Francisco de Vitoria, Ctra. M-515 Pozuelo-Majadahonda Km. 1,800; 28223, Pozuelo de Alarcón (Madrid), o al correo electrónico dpd@ufv.es

Relativo al paciente

D./D.ª…………………………………………………………...con
D.N.I………………………

He sido suficientemente informado del procedimiento que se me va a realizar, explicándoseme sus riesgos, complicaciones y alternativas, lo he comprendido y he tenido el tiempo suficiente para valorar mi decisión. Por tanto, estoy satisfecho con la información recibida. Por ello, doy mi consentimiento para que se me realice dicho procedimiento por el médico responsable. Mi aceptación es voluntaria y puedo revocar este consentimiento cuando lo crea oportuno, sin que esta decisión repercuta en mis cuidados posteriores.

Sé que estoy siendo atendido en un Hospital Universitario AUTORIZO SI NO para la utilización de imágenes e información de la Historia Clínica resultante del procedimiento con fines docentes o científicos, tratándose de forma confidencial y anónima según dispone la legislación vigente.

Firma del paciente Fecha ……/…../……….

Relativo al médico

Dr./Dra., he informado al paciente y/o tutor o familiar del objeto y naturaleza del procedimiento que se le va a realizar, explicándole los riesgos, complicaciones y alternativas posibles.

Firma del médico Fecha ……/……/……….

Relativo a los familiares y tutores

El paciente D./D. ª no tiene capacidad para decidir en este

momento.

D./D.ª……………………………………………………… con D.N.I… y en calidad de

… he sido informado/a suficientemente del procedimiento que se le va a

realizar. Por ello, doy expresamente mi consentimiento. Mi aceptación es voluntaria y puedo retirar este consentimiento cuando lo crea oportuno.

7.5. FUNDING

This doctoral thesis is conducted under the supervision of Dr. Juan Pablo Romero Muñoz, who has been contributing to the financial support essential for this scholarly endeavor. The funding for this research project has been provided through Grant PID2020-113222RB-C21, awarded by the Ministry of Science and Innovation (MCIN) and the Spanish State Research Agency (AEI), under the auspices of the Spanish government (10.13039/501100011033). This grant has played a pivotal role in facilitating the comprehensive investigation and analysis required for the completion of this thesis.

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8. ATTACHMENTS

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Review Article

Repetitive Transcranial Magnetic Stimulation of Primary Motor Cortex for stroke upper limb motor sequelae rehabilitation: A systematic review

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Abstract.
BACKGROUND: Repetitive Transcranial Magnetic Stimulation (rTMS) over the primary motor cortex (M1) has been used to treat stroke motor sequelae regulating cortical excitability. Early interventions are widely recommended, but there is also evidence showing interventions in subacute or chronic phases are still useful.
OBJECTIVE: To synthetize the evidence of rTMS protocols to improve upper limb motor function in people with subacute and/or chronic stroke.
METHODS: Four databases were searched in July 2022. Clinical trials investigating the effectiveness of different rTMS protocols on upper limb motor function in subacute or chronic phases post-stroke were included. PRISMA guidelines and PEDro scale were used.
RESULTS: Thirty-two studies representing 1137 participants were included. Positive effects of all types of rTMS protocols on upper limb motor function were found. These effects were heterogeneous and not always clinically relevant or related to neurophysiological changes but produced evident changes if evaluated with functional tests.
CONCLUSION: rTMS interventions over M1 are effective for improving upper limb motor function in people with subacute and chronic stroke. When rTMS protocols were priming physical rehabilitation better effects were achieved. Studies considering minimal clinical differences and different dosing will help to generalize the use of these protocols in clinical practice.

Keywords: Repetitive transcranial magnetic stimulation, stroke, upper limb motor function, subacute, chronic, motor cortex

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1. Introduction

According to World Health Organization, 15 million people suffer from stroke each year, which is also the leading cause of adult disability worldwide (Katan & Luft, 2018). Stroke modifies the functioning of several neurological areas. The most common and the one that causes the main limitations in basic activities of daily life is the motor domain (Hankey et al., 2002). Total recovery of motor function occurs in less than 40% of survivors, despite rehabilitation programs (Hummel & Cohen, 2006).

Brain plasticity plays an important role in the recovery of lost functions in the brain and is achieved through the reorganization of networks (Carino-Escobar et al., 2019; Dromerick et al., 2021; Hordacre et al., 2021). Although there is evidence that brain plasticity still operates at 4 months from stroke (Cicinelli et al., 1997) more than 60% of subjects with hand function impairment will not reach sufficient functional levels to be independent in their Basic Activities of Daily Living after 6 months (Dobkin, 2004).

Cortical excitability diminishes after a stroke in the lesioned hemisphere (Pennisi et al., 1999), reflected by the decrease of motor-evoked potentials (MEPs) in response to transcranial magnetic stimulation (TMS). Additionally, decreased cortical excitability after stroke produces changes in movement kinematics (Ameli et al., 2009), which in turn alter upper limb motor function. Cortical excitability can be measured by determining the intensity required of a single TMS pulse applied to the primary motor cortex (M1) to evoke a motor response. This neurophysiological measure has been interpreted as a prognostic factor of upper limb functional recovery (Bembenek et al., 2012).

In recent years, several non-invasive neuromodulation techniques have been shown to be effective to enhance brain plasticity and stroke recovery. Among these interventions, repetitive TMS (rTMS) is a technique that involves repetitive pulses of magnetic stimulation applied focally in a particular brain area at a specific frequency (Kobayashi & Pascual-Leone, 2003). Frequencies of rTMS equal to or less than 1 Hz provoke an inhibitory effect, while higher frequencies can increase cortical excitability and cause facilitation (Rossi et al., 2009). Cortical excitability can be modulated through rTMS, inducing synaptic plasticity, decreasing maladaptive mechanisms and inducing normal physiological activity (Xiang et al., 2019), what might underly positive clinical effects according to recent evidence-based guidelines (Lefaucheur et al., 2020).

Although promising results have been reported from the use of rTMS in upper limb motor rehabilitation after stroke, standardized protocols have not been widely validated, and most of the protocols are applied in early stages post stroke (< 6 months) (G. Chen et al., 2022; Lefaucheur et al., 2020). In the last years, its interest has increased by combining it with conventional rehabilitation programs, mirror therapy (Kim et al., 2016) or motor training (Kwon et al., 2014). In fact, combining rTMS protocols with other neurorehabilitation therapies might target different mechanisms and might be desirable to potentiate homeostatic metaplasticity, creating a synergistic effect. The synergistic effects that occur might vary depending on the type of application and dose of rTMS, as well as rehabilitation dosage and content (Takeuchi & Izumi, 2015).

Early post stroke rehabilitation studies suggest the existence of a period of high plasticity in which the patient seems to be more responsive to treatment, nevertheless patients with persisting deficits in subacute (3–6 months) and chronic (> 6 months) stages continue rehabilitation, and their therapists look for adjuvant therapies to optimize results. There is scarce information about the effects of rTMS neuromodulation on upper limb motor function during subacute and chronic stages.

The aim of this review is to synthetize the available evidence on the effects of rTMS to enhance upper limb motor function in subacute and chronic stages after stroke. The updated information on the treatment strategies and results will provide an entry point for new studies that seek unified protocols that can be applied clinically to impact the recovery process of subacute and chronic stroke upper limb motor sequelae.

2. Methods

This review followed the PRISMA 2020 guidelines (Page et al., 2021) and was prospectively preregistered on the Open Science Framework (https://osf.io/bnqd7/).

2.1. Identification: search strategy and sources

Four electronic databases were searched: Web of Science, PubMed, Scopus and PEDro. Citation track-

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ing from eligible articles was carried out manually. The search was performed on July 13th, 2022.

Search terms were variations on three concepts: rTMS, upper limb motor function and stroke. Search strings varied slightly depending on the MeSH terms within each database. The following terms were selected: (rTMS OR "repetitive transcranial magnetic stimulation" OR "Theta Burst") AND (stroke OR "cerebrovascular accident" OR CVA) AND "motor cortex" AND (upper limb or hand) with no time limits.

2.2. Eligibility criteria and Selection

The eligibility criteria were as follows. Inclusion criteria: (1) peer-reviewed article, (2) written in English, French or Spanish, (3) clinical trial design (including parallel and crossover trials), (4) intervention with rTMS on M1, of any type (unilateral/bilateral, high/low frequency, theta burst), (5) participants who had suffered an ischemic or hemorrhagic stroke and were in the subacute (3–6 months) or chronic (> 6 months) recovery periods and (6) upper limb motor function outcomes provided. Exclusion criteria: (1) protocols for randomized trials, pilot, or observational studies, (2) other non-invasive (transcranial direct current stimulation) and invasive (epidural electrical stimulation) brain stimulation interventions, (3) studies investigating animal models of stroke or pediatric stroke, (4) upper limb outcome measure not provided.

After removing duplicates, search results were double screened on title and abstract by two authors (F.S.C and J.P.R). Proceedings, conference articles, book chapters, posters and editorials were excluded before full text screening. Any disagreement was resolved through consensus and if it was not reached, a third author (Y.G.Z) had the deciding vote.

2.3. Data extraction and analysis

Once the selection criteria were applied, full-text articles were screened to collect information for each study based on a data extraction table developed in Microsoft Excel. This table covered information about study characteristics (year of publication, design, purpose, presence of a control group), sample size, participant characteristics (age, gender, type of stroke (ischemic or hemorrhagic), lesion location (cortical or subcortical) and evolution phase (subacute or chronic)), intervention dosage (total number of sessions and frequency (sessions per week)), rTMS parameters (frequency (in Hz), intensity (percentage of the action/resting motor thresholds) and number of total pulses), outcome measures of upper limb motor function and main findings.

2.4. Methodological quality assessment

The PEDro scale was used (Maher et al., 2003; Sherrington et al., 2000). It comprises 11 items whose purpose is to quickly identify which clinical trials may have sufficient internal validity and enough statistical information to make their results interpretable. Each item is rated as 1 point (criterion fulfilled) or 0 points (criterion not fulfilled) and therefore higher scores imply greater methodological quality. The first item of the scale (source of participants and selection criteria) is not given a numeric value and thus the instrument provides scores between 0–10 points.

Each article was assessed by 2 independent evaluators (F.S.C. and Y.G.Z.). To measure the consensus between both raters the kappa value (k) was calculated, and interpreted as high, moderate and low level of agreement when k was > 0.7, 0.5–0.7 and < 0.5, respectively.

3. Results

3.1. Study selection

The PRISMA diagram (Fig. 1) reflects the flow of information. Initially, the searches yielded 1164 results, 559 of which were duplicates, and thus, were eliminated. The remaining 605 records were screened on titles and abstracts. After this step, 504 records were removed, leaving a total of 101 full texts that were screened for eligibility. Of these, 69 articles were excluded (see Fig. 1). A total of 32 articles met the selection criteria and were finally included.

3.2. Study characteristics

The review included 32 studies, 24 with parallel group design and 8 with crossover design. Thirty studies randomly allocated participants to interventions and 2 did not. Twenty-nine studies presented a control group and 3 did not (Table 1).

3.3. Participant characteristics

A total sample of 1137 participants (711 men, mean age $= 59.5 \pm 10.4$ years) were included. They

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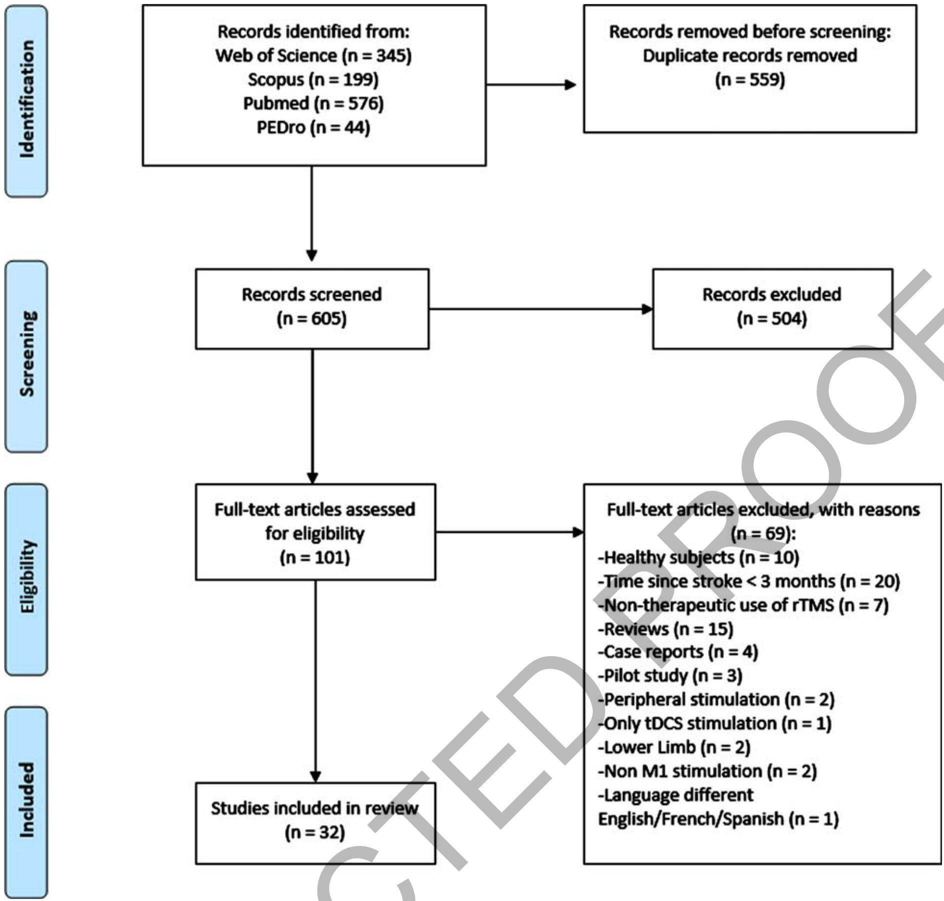

Fig. 1. PRISMA flow diagram illustrating the selection process and flow of information.

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represent the reality of the sociodemographic characteristics of people with stroke. Twenty-three studies included participants with ischemic and hemorrhagic stroke, and 9 studies included only people with ischemic stroke. Twenty-seven studies enrolled participants who had suffered a cortical or subcortical lesion, and 5 studies included only people with subcortical lesions. Twenty-six studies enrolled participants in the chronic stage and 6 in the subacute phase.

3.4. *rTMS and control interventions*

3.4.1. *rTMS interventions*

In terms of stimulation frequency, 73% of the studies used low-frequency rTMS on the contralateral M1 compared to 16% of them, which used high-frequency rTMS applied to the ipsilateral M1(Cho et al., 2017; Haghighi et al., 2021; Kim et al., 2016; Kwon et al., 2014; Malcolm et al., 2007). Only 20% of the studies used intermittent TBS to stimulate the ipsilateral M1 (Ackerley et al., 2010; Chen et al., 2021; Sung et al., 2013; Talelli et al., 2012; Wang et al., 2014; Zhang et al., 2022) (Table 2). The most common frequencies used were 1 Hz for low frequency, inhibitory rTMS, and 10 Hz for high-frequency, facilitatory protocols. The number of total pulses varied between 160 and 2400. Fifteen studies used more than 1000 pulses. Regarding the stimulation threshold, just three studies used 100% of the resting motor threshold (Demirtas-Tatlidede et al., 2015; Fregni et al., 2006; Rose et al., 2014) and the rest used 90–80%.

3.4.2. *Control interventions*

Twenty-one studies used sham TMS as the control intervention. Five trials used conventional rehabilitation and one study used audiotape, a tape-guided relaxation program with soothing music. Two studies used rTMS as control intervention, when comparing to rTMS plus adjuvant therapy and one study utilized

Table 1
Characteristics of the included studies

Study	Design	Objective	Sample size (Mean ± SD Age, years)	Gender (F/M)	Control Group	Lesion Location /Stroke type	Stroke Evolution Time
(Du et al., 2022)	Randomized, single-blinded, controlled trial.	To investigate the effect of neuromuscular electrical stimulation (NMES) combined with rTMS on poststroke hemiparetic upper limb rehabilitation.	240 (58 ± 36.2)	116/124	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Zhang et al., 2022)	Randomized, single-blinded, controlled trial.	To investigate the effects of priming iTBS followed by iTBS + robot-assisted training on poststroke hemiparetic upper limb recovery	42 (61 ± 7.7)	18/24	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Yamada et al., 2022)	Open-label, crossover, controlled study.	To compare the effects of the Program NEURO (rTMS + Occupational therapy) in patients with hemiparesis after stroke	37 (63 ± 8.1)	14/23	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Kuzu et al., 2021)	Randomized, double blinded, and controlled clinical trial.	To examine the effect of cTBS and low frequency rTMS on upper extremity spasticity and functional recovery in stroke patients.	20 (60.8 ± 8.6)	8/12	Yes	Cortical-Subcortical/Ischemic	Chronic
(Chen et al., 2021)	Randomized, single-blinded, controlled trial	To investigate the additive effect of iTBS on virtual reality-based cycling training (VCT) for upper limb function after stroke	23 (52 ± 9.8)	5/18	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Subacute
(Haghighi et al., 2021)	Randomized, parallel, double-blinded controlled trial	To determinate the effects of a rehabilitation program (RP) in conjunction with rTMS on paretic upper limb function after stroke	20 (52 ± 11.4)	9/11	Yes	Subcortical/ Ischemic-Hemorrhagic	Subacute
(Pan W; Wang P; Song X; Sun X; Xie Q, 2019)	Randomized, single-blinded, controlled trial.	To investigate the effects of low frequency rTMS stimulation combined with motor imagery (MI) on upper limb function after stroke.	42 (63 ± 5.2)	14/28	Yes	Cortical-subcortical/ Ischemic	Subacute
(Harvey et al., 2018)	Randomized, multicenter, blinded, sham-controlled trial.	To determine the effects of low-frequency electric field navigated rTMS to non-injured M1 improve arm motor function in hemiplegic stroke patients when combined with motor training.	199 (58.7 ± 13.1)	69/130	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Subacute/ Chronic
(Johnson et al., 2018)	Randomized, open label, controlled trial study	To evaluate the efficacy of combined rTMS + BCI, compared to sham rTMS + BCI, on motor recovery after stroke.	3 (55 ± 13)	2/1	Yes	Subcortical/Ischemic	Chronic

(*Continued*)

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Table 1
(*Continued*)

Study	Design	Objective	Sample size (Mean ± SD Age, years)	Gender (F/M)	Control Group	Lesion Location /Stroke type	Stroke Evolution Time
(Aşkın et al., 2017)	Single-blinded, randomized controlled study.	To assess the efficacy of inhibitory rTMS in healthy hemisphere M1 on upper extremity motor recovery and functional outcomes in chronic ischemic stroke patients.	40 (58 ± 11.7)	11/29	Yes	Cortical-subcortical/ Ischemic	Chronic
(Cho et al., 2017)	Randomized, open label, parallel study.	To investigate the effect of simultaneous bilateral stimulation using rTMS and tDCS over the bilateral M1 for the recovery of motor function.	30 (59 ± 10.6)	13/17	Yes	Cortical-Subcortical/Ischemic-Hemorrhagic	Subacute
(Bashir et al., 2016)	Open-label, controlled study.	To compare the effects of navigated rTMS between stroke patients and control subjects.	16 (62 ± 11.1)	8/8	No	Cortical-Subcortical/Ischemic-Hemorrhagic	Chronic
(Cassidy et al., 2015)	Randomized, crossover, single-blinded design	To evaluate changes in cortical excitability and paretic hand function in chronic stroke following three types of primed low frequency rTMS treatments on healthy hemisphere M1.	11 (64 ± 9.4)	3/8	Yes	Cortical-Subcortical/Ischemic-Hemorrhagic	Chronic
(Demirtas-Tatlidede et al., 2015)	Open-label, single-arm study.	To investigate the safety of contralesional M1 rTMS in a selected group of severe chronic stroke patients.	10 (59.5 ± 11)	6/4	No	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Wang et al., 2014)	Randomized, double-blinded, controlled, parallel study.	To test the long-term efficacy of different sequences of 1 Hz rTMS on the healthy hemisphere M1 with contralateral iTBS to identify the strategy producing the most intense and long lasting electrophysiological / motor improvements in the paretic hand.	48 (62.6 ± 12.5)	10/38	Yes	Cortical-subcortical/ Ischemic	Subacute
(Rose et al., 2014)	Randomized, double-blinded, controlled study.	To assess the effects of low frequency rTMS in healthy hemisphere M1 as an adjuvant to functional task practice, to improve upper extremity function.	19 (64.7 ± 8.0)	6/13	Yes	Cortical-subcortical/ Ischemic	Chronic
(Kwon et al., 2014)	Randomized, single-blinded, crossover study.	To determinate the most effective method for combining rTMS and motor training in stroke patients.	14 (53 ± 12.4)	3/11	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Barros Galvão et al., 2014)	Randomized, double-blinded, controlled trial.	To assess the efficacy of inhibitory rTMS for decreasing upper-limb muscle tone after chronic stroke.	20 (61 ± 9.4)	7/13	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Carey et al., 2014)	Randomized, single-blinded, controlled trial.	To analyse the characteristics of responder vs. non responders receiving rTMS to improve hand function after stroke.	12 (69 ± 10.1)	4/8	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Etoh et al., 2013)	Randomized double-blinded crossover study	To investigate whether multiple sessions of 1-Hz rTMS on healthy hemisphere M1 facilitates the effect of repetitive facilitation exercises on hemiplegic upper-limb function in chronic stroke patients.	18 (59.7 ± 11)	4/14	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic

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(Tretriluxana et al., 2013)	Randomized, single-blinded, controlled, crossover study	To investigate the effect of inhibitory low frequency rTMS applied to the non-lesioned hemisphere on kinematics and coordination of paretic arm reach-to-grasp action.	9 (59 ± 6.8)	4/5	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Sung et al., 2013)	Randomized, single-blinded, controlled study.	To investigate the potential for a consecutive suppressive-facilitatory rTMS protocol to improve motor outcomes after chronic stroke.	54 (63 ± 12.1)	13/41	Yes	Cortical Subcortical / Ischemic-Hemorrhagic	Chronic
(Avenanti et al., 2012)	Randomized, double-blinded, controlled, study.	To investigate the long-term behavioural and neurophysiologic effects of rTMS on healthy hemisphere M1 combined with physical therapy intervention in chronic stroke patients with mild motor disabilities.	30 (63 ± 9.53)	14/16	Yes	Cortical-Subcortical / Ischemic-Hemorrhagic	Chronic
(Talelli et al., 2012)	Semi-randomized, single-blinded, controlled trial.	To explore whether long-lasting clinically important gains can be achieved by adding TBS to a rehabilitation program for the hand.	41 (56 ± 13.7)	20/21	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Ackerley et al., 2010)	Double-blinded, controlled, crossover study.	To determine the effects of M1 TBS and standardized training on upper-limb function of patients with chronic stroke.	10 (60 ± 11)	7/3	Yes	Subcortical/ Ischemic-Hemorrhagic	Chronic
(Takeuchi et al., 2009)	Randomized, double-blinded, study.	To investigate whether bilateral rTMS stimulating injured M1 and inhibiting healthy M1 may improve the paretic hand in patients after stroke.	30 (59 ± 12.4)	8/22	No	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Takeuchi et al., 2008)	Randomized, double-blinded, controlled study.	To investigate whether 1 Hz TMS on healthy hemisphere M1 improve the motor learning of the affected hand in patients after stroke.	20 (62.3 ± 8.4)	4/16	Yes	Subcortical/Ischemic	Chronic
(Malcolm et al., 2007)	Randomized, double-blinded, controlled study.	To test the potential adjuvant effect of rTMS on motor learning in a group of survivors undergoing constraint-induced therapy (CIT) for upper limb hemiparesis.	19 (67 ± 6.8)	8/11	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Fregni et al., 2006)	Randomized, single-blinded, controlled study	To evaluate whether five sessions of low frequency rTMS on healthy hemisphere M1 produces improvements in upper limb motor function and whether this approach is safe and its effects durable	15 (56 ± 11.5)	4/11	Yes	Cortical-subcortical/ Ischemic	Chronic
(Kim et al., 2006)	Pseudorandomized, crossover, controlled study.	To investigate high-frequency rTMS-induced cortical excitability and the associated motor skill acquisition in chronic stroke patients.	15 (54 ± 4.5)	2/13	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic
(Takeuchi et al., 2005)	Randomized, double-blinded, controlled study.	To study if 1 Hz rTMS on healthy hemisphere M1 improves motor performance of the affected hand in stroke patients by decreasing the transcallosal inhibition	20 (59.0 ± 9.6)	5/15	Yes	Subcortical/Ischemic	Chronic
(Mansur et al., 2005)	Double-blinded, controlled, crossover, study.	To investigate the use of low frequency rTMS on the unaffected hemisphere to decrease interhemispheric inhibition of the lesioned hemisphere and improve motor function in patients after stroke.	10 (53 ± 18.1)	7/3	Yes	Cortical-subcortical/ Ischemic-Hemorrhagic	Chronic

Note: subacute stage: 3–6 months post-stroke; chronic stage: > 6 months post-stroke. Acronyms: rTMS: Repetitive Transcranial Magnetic Stimulation; iTBS: Intermittent Theta Burst; cTBS: Continuous Theta Burst Stimulation; M1: Primary Motor Cortex.

Table 2

Characteristics of the included studies according to dosage, transcranial magnetic stimulation parameters, outcome measures and main findings

Study	Stimulation parameters		Outcome Measures	Main Finding
	Number of sessions	Frequency, Intensity and Pattern		
(Du et al., 2022)	20 (5 times /week × 4 weeks)	**Group A:** Control group, only routine treatment. **Group B**: NMES based on rehabilitation treatment. **Group C**: rTMS based on rehabilitation treatment (cM1, 1 Hz, 20 min, 1200 pulses; 90% RMT) **Group D**: NMES + rTMS (cM1, 1 Hz, 20 min, 1200 pulses; 90% RMT) based on routine treatment.	-Modified Barthel Index (MBI). -FMA-UE and MAS. - MEPs and Central motor conduction time (CMCT)	Group D showed statistically significant improvements ($p < 0.05$) in FMA, MAS, MEPs and CMCT.
(Zhang et al., 2022)	10 (5 times /week × 2 weeks)	**Group A:** Priming iTBS (iM1 cTBS at 70% RMT), followed by iTBS at 70% RMT + robot-assisted training. **Group B:** Nonpriming iTBS (iM1 cTBS at 20% RMT) followed by iTBS at 70% RMT + robot-assisted training. **Group C**: Sham stimulation (iM1 cTBS at 20% RMT) followed by iTBS at 20% RMT immediately before robot-assisted training.	-FMA-UE, ARAT. -The mean velocity of movement. -EEG.	Group A presented greater improvement than Group B and C in FMA-UE, ARAT, and mean velocity of movement. Only group A showed statistically significant differences in FMA-UE ($p < 0.05$)
(Yamada et al., 2022)	10 (5 times /week × 2 weeks)	**Group A:** Neuro Program (cM1 1 Hz rTMS (40 min, 2400 pulses; 90% RMT) followed by Occupational Therapy) **Group B:** Occupational Therapy	-FMA-UE, WMFT.	Group A showed statistically significant improvements ($p < 0.05$) in FMA and WMFT.
(Kuzu et al., 2021)	10 (5 times /week × 2 weeks)	**Group A:** Active cM1 1Hz rTMS (20 min, 1200 pulses; 90% RMT) followed by Physical Therapy. **Group B:** Active cM1 cTBS (600 pulses; 80% AMT) followed by Physical Therapy. **Group C:** Sham cM1 cTBS (600 pulses; 80% AMT) followed by Physical Therapy	-FMA-UE and MAS. -FIM, MAL and Brunnstrom stage.	Group A and B showed significant improvements in FMA-UE, FIM and MAL ($p < 0.05$), and non-significantly in MAS ($p > 0.05$). Group C did not present significant improvements in any variable.
(Chen et al., 2021)	15 (5 times/week × 3 weeks)	**Group A:** Active iM1 iTBS (80% AMT) followed by 60 min of virtual reality-based cycling training (VCT) **Group B:** Sham iM1 iTBS (80% AMT) followed by 60 min of virtual reality-based cycling training (VCT)	-FMA-UE, MAS-UE, ARAT, NHPT, BBT, MAL and SIS.	Group A presented greater improvement than Group B in MAS-UE, MAL, and SIS ($p < 0.05$).
(Haghighi et al., 2021)	10 (3 times/week)	**Group A:** Active iM1 20 Hz rTMS (2000 pulses; 90% RMT) followed by a Rehabilitation Program (RP) **Group B:** Rehabilitation Program	-FMA, BBT. -Grip strength and pinch strength.	All outcome measure showed significant improvements ($p < 0.05$) regarding group A.

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(Pan W; Wang P; Song X; Sun X; Xie Q, 2019)	10 (5 times /week × 2 weeks)	**Group A:** Active cM1 1 Hz rTMS (1500 pulses; 90% RMT) followed by an Audio-Based Motor Imagery (30 min) **Group B:** Active cM1 1 Hz rTMS (1500 pulses; 90% RMT) followed by an Audiotaped-Led Relaxation (30 min)	-FMA-UE, WMFT, BBT and MBI.	They found greater change of FMA-UE, WMFT, BBT and MBI scores in group A ($p<0.05$).
(Harvey et al., 2018)	18 (3 times/week × 6 weeks)	**Group A:** Active cM1 1 Hz rTMS (> 15minutes) followed by 60 min of task-oriented rehabilitation therapy. **Group B:** Sham cM1 1 Hz rTMS (> 15minutes) followed by 60 min of task-oriented rehabilitation therapy.	-FMA-UE, ARAT and WMFT.	All outcome measures showed significant improvement in both groups ($p<0.05$). Significant differences between groups were not found.
(Johnson et al., 2018)	18 (3 times / week × 6 weeks)	**Group A:** Active cM1 1Hz rTMS (10 min; 90% RMT) followed by BCI training (First 3 weeks) + BCI training (Second 3 weeks) **Group B:** Sham cM1 1Hz rTMS (10 min; 90% RMT) followed by BCI training (First 3 weeks) + BCI training (Second 3 weeks)	-BBT, FTT. -IHI, MEP -PVC	Increased ipsilesional motor activity and improvements in BBT and FTT for group A ($p<0.05$)
(Aşkın et al., 2017)	10 (5 times /week × 2 weeks)	**Group A:** Physical therapy **Group B:** Active cM1 1 Hz rTMS (1200 pulses, 90% RMT) followed by Physical therapy	-MAS, Brunnstrom Stages, FMA, BBT. -FIM, FAS. -MMSE.	Regarding group B, there were statistically significant improvements in all clinical outcome measures except for the Brunnstrom Stage. MMSE scores were significantly increased in group B.
(Cho et al., 2017)	10 (5 times /week × 2 weeks)	**Group A:** Active iM1 10 Hz rTMS (20 min, 1000 pulses, 90% RMT) with the simultaneous application of cathodal tDCS (2mA) over cM1. **Group B:** Active iM1 10 Hz rTMS (20 min, 1000 pulses, 90% RMT)	-FMA-UE -FMA-LE -FMA-T	Significant differences were found in FMA-UE and FMA-T in the dual mode stimulation group ($p<0.05$)
(Bashir et al., 2016)	1	**Patient Group:** Active cM1 1 Hz rTMS (1200pulses, 90% RMT) **Healthy Group:** Active right M1 1 Hz rTMS (1200pulses, 90% RMT)	-FTT, NHPT, Strength Index, Reaction time. -RMT, MEP, ICI, IF, CSP.	All participants improved FTT, strength index and increased excitability, but group A was more significative ($p<0.05$)
(Cassidy et al., 2015)	1	**Group A:** Active cM1 6 Hz rTMS priming (10 min, 600 pulses, 90%RMT) + active cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT). **Group B:** Active cM1 1 Hz rTMS priming (10 min, 600pulses, 90%RM) + active cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT). **Group C**: Sham cM1 6 Hz rTMS priming (10 min, 600 total pulses, 90%RMT) + active cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT).	- RMT, SICI, IF and CSP. - MEP. - BBT.	No significant differences were found in outcome measure of group B and C. Regarding group A, there are significant improvements in: -Ipsilesional CSP ($p<0.05$) -SICI from baseline ($p<0.05$) -BBT ($p<0.05$)

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Table 2
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Study	Stimulation parameters		Outcome Measures	Main Finding
	Number of sessions	Frequency, Intensity and Pattern		
(Demirtas-Tatlidede et al., 2015)	10 (5 times /week × 2 weeks)	Active cM1 1 Hz rTMS (1600 pulses, 100% RMT)	- FMA, hand grip strength, MAS and WMFT. - MEP, SICI, ICF and TCI.	No significant differences were found in MAS, WMFT, MEP, SICI, ICF and TCI duration Significant differences were found in FMA and hand grip strength ($p < 0.05$). Both improve compared to the previous evaluation. Changes persisted at follow up 1 month
(Rose et al., 2014)	16 (4 times /week × 4 weeks)	**Group A:** Active cM1 1 Hz rTMS (1200 pulses, 100% RMT) followed by 1 hour of functional task practice activities. **Group B:** Sham cM1 1 Hz rTMS (1200 pulses, 100% RMT) followed by 1 hour of functional task practice activities.	-WMFT, FMA, MAS, MAL, ARAT, LLFDI and ROM. -Grip force, Lateral Pinch force, Palmar Pinch force, and 3-Jaw Chuck Force. -Light Touch Sensation. -RMT, SICI, V50. -Movement time, trunk displacement and maximum resultant velocity.	No significant differences were detected for any of the clinical measures between the real-rTMS and sham-rTMS group. *Post hoc* small but statistically significant improvements in upper extremity behavioural measures. No significant differences were detected for SICI, V50 or RMT ($p > 0.05$).
(Kwon et al., 2014)	1	**Group A:** Active iM1 10 Hz rTMS (20 min, 1000 pulses, 90% RMT) and simultaneous finger motor task during each inter-train interval. **Group B:** Active iM1 10 Hz rTMS (10 min, 1000 pulses, 90% RMT) and after stimulation, finger motor task.	-Movement accuracy and movement time. -Purdue Pegboard Test and NHPT.	All participants improved all outcome measure, but there isn't significant difference between both groups.
(Wang et al., 2014)	20 (5 times /week × 4 weeks)	**Group A:** 10 sessions Active cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT) followed by 10 sessions Active iM1 iTBS (190 seconds, 600 pulses, 80% RMT) + Conventional physiotherapy. **Group B**: 10 sessions Active iM1 iTBS (190 seconds, 600 pulses, 80% RMT), followed by 10 sessions Active cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT) + Conventional physiotherapy. **Group C:** 10 sessions Sham cM1 1 Hz rTMS (10 min, 600 pulses, 90% RMT) followed by 10 sessions Sham iM1 iTBS (190 seconds, 600 pulses) + Conventional physiotherapy	- FMA, WMFT, and MRC. - MEP.	No significant differences were found in outcome measure of group B and C. Regarding group A, there are significant improvements in: - MRC ($P < 0.05$) - FMA ($P < 0.05$) - WMFT ($P < 0.05$) The effects lasted at least 3 months

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(Barros Galvão et al., 2014)	10 (3 times/week)	**Group A:** Active cM1 1 Hz rTMS (1500 pulses; 90% RMT) followed by Physical therapy. **Group B:** Sham cM1 1 Hz rTMS (1500 pulses; 90% RMT) followed by Physical therapy.	-MAS, FMA, FIM. -SSQOL. -ROM.	In the group A, 90% of the patients at postintervention and 55.5% at 1 month follow-up showed a decrease of < 1 in the MAS score, representing clinically important differences. There was no difference in the number of participants who showed clinically relevant changes between groups, except MAS.
(Carey et al., 2014)	5 (5 times/week)	**Group A:** Active cM1 6Hz (10min, 600pulses, 90% RMT) + Active cM1 1Hz (10 min, 600 pulses, 90% RMT) followed by tracking training. **Group B:** Active cM1 6Hz (10min, 600pulses, 90% RMT) + Active cM1 1Hz (10 min, 600 pulses, 90% RMT).	-TEMPA -NIHSS -FMA-UE, MMSE.	Results showed significant differences only in TEMPA ($p < 0.05$)
(Etoh et al., 2013)	20 (5 times /week × 4 weeks)	**Group A:** Active cM1 1Hz rTMS (4 min, 240 pulses, 90% RMT) for 2 weeks + Sham 5cm posterior to cM1 1Hz rTMS (4 min) for 2 weeks + repetitive facilitation exercises for 40 min during active and sham rTMS sessions **Group B:** Sham 5cm posterior to cM1 1Hz rTMS (4 min) for 2 weeks + Active cM1 1Hz rTMS (4 min, 240 pulses, 90% RMT) for 2 weeks + repetitive facilitation exercises for 40 min during active and sham rTMS sessions	-FMA, ARAT, MAS and STEF.	Significant differences were found in ARAT, FMA and STEF scores when motor facilitation was performed during active but not sham rTMS. No significant changes were found in MAS.
(Tretriluxana et al., 2013)	2	**Group A:** Reach to grasp task followed by active cM1 1Hz (20 min, 1200 pulses, 90%RMT). **Group B:** Reach to grasp task followed by sham cM1 1Hz (20 min, 1200 pulses, 90%RMT).	-Peak transport velocity, peak aperture, time of peak transport velocity, time of peak aperture and total movement time. -MEP.	Significant reduction in the MEP amplitude between groups ($P < 0.05$). There were no changes in Reach to Grasp kinematics.
(Sung et al., 2013)	20 (5 times /week × 4 weeks)	**Group A:** Active cM1 1Hz followed by active iTBS over iM1. **Group B:** Sham cM1 1Hz followed by active iTBS over iM1. **Group C:** Active cM1 1Hz followed by sham iTBS over iM1. **Group D:** Sham cM1 1Hz followed by sham iTBS over iM1.	-FMA-UE, WMFT. -MRC -Reaction Time and FTT -MEP	Only group A showed greater muscle strength (MRC), FMA-UE, WMFT and Reaction times in comparison with the other 3 groups ($p < 0.05$).
(Avenanti et al., 2012)	10 (daily sessions)	**Group A:** Active cM1 1 Hz rTMS (25 min, 1500 pulses, 90% RMT) **Group B**: Sham cM1 1 Hz rTMS (25min, 1500 pulses) Each protocol divided into 2 groups: stimulation before PT and stimulation after PT	-Tip-pinch, and power-grip force. - ISP, RMT. - JHFT, NHPT and BBT.	Significant differences were found in the outcome measures (JTT, NHPT, BBT and key grip) on both rTMS groups with bigger and longer lasting effects in the rTMS- PT group. iSP and rMT changed significantly in both rTMS groups. Effects persisted for 90 days

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Table 2
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Study	Stimulation parameters		Outcome Measures	Main Finding
	Number of sessions	Frequency, Intensity and Pattern		
(Talelli et al., 2012)	10 (5 times /week × 2 weeks)	**Group A:** Active iM1 iTBS at 80% AMT followed by physical therapy. **Group B:** Sham iM1 iTBS at 80% AMT followed by physical therapy. **Group C:** Active cM1 cTBS at 80% AMT followed by physical therapy. **Group D:** Sham iM1 cTBS at 80% AMT followed by physical therapy.	-NHPT, JTT. -Grip and pinch-grip dynamometry.	There were no differences between the active treatment and sham groups in any of the outcome measure.
(Ackerley et al., 2010)	1	**Group A:** Active iM1 iTBS at 90% AMT followed by motor training. **Group B:** Active cM1 cTBS at 90% AMT followed by motor training. **Group C:** Sham TBS (to either M1) followed by motor training.	-Movement accuracy and movement time. -ARAT. -MEP.	Training after real TBS (Group A/B) improved paretic-hand grip-lift kinetics. ARAT improved in Group A but not in Group B, this was correlated with reduced ipsilesional corticomotor excitability.
(Takeuchi et al., 2009)	10 (5 times /week × 2 weeks)	**Group A:** Active cM1 1 Hz rTMS (1000 pulses, 90% RMT) + motor training after stimulation. **Group B:** Active iM1 10 Hz rTMS (1000 pulses, 90% RMT) + motor training after stimulation. **Group C:** Active cM1 1 Hz rTMS (1000 pulses) + Active iM1 10 Hz rTMS (1000 pulses, 90% RMT) + motor training after stimulation.	-ICI, RMT and MEP. -Acceleration and Pinch Force.	No significant improvement was found in outcome measure of group A and B. Regarding group C, there are significantly better outcomes in: -Acceleration ($p < 0.05$) -Pinch force ($p < 0.05$) -MEP (contralesional $p < 0.05$, ipsilesional $p < 0.05$) - ICI ($p < 0.05$)
(Takeuchi et al., 2008)	1	**Group A:** Active cM1 1 Hz rTMS (25 min, 1500 pulses, 90% RMT) + Motor training **Group B:** Sham cM1 1 Hz rTMS (25 min, 1500 pulses) + Motor training	-ICI, RMT and MEP. -Acceleration and Pinch Force.	In group A significant improvements were found in Acceleration and Pinch force. Contralesional and ipsilesional MEP increased ($p < 0.05$). No significant differences were found in RMT.
(Malcolm et al., 2007)	10 (5 times /week × 2 weeks)	**Group A:** Active iM1 20 Hz rTMS (2000 pulses, 90% RMT) + Constraint-Induced Therapy (CIT) **Group B:** Sham iM1 20 Hz rTMS (2000 pulses, 90% RMT) + Constraint-Induced Therapy (CIT)	-WMFT, MAL and BBT. -RMT/AMT.	Group A presented significant improvements in WMFT, MAL and BBT ($p < 0.05$). There was no significant difference in motor threshold between the groups.

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(Fregni et al., 2006)	5 (daily sessions)	**Group A:** Active cM1 1Hz rTMS (20 min, 1200 pulses, 100% RMT) **Group B:** Sham cM1 1Hz rTMS (20 min, 1200 pulses)	-CRT, SRT, JHFT and PPT. - MMSE, Stroop test and digit span forward and backward. -RMT.	No significant differences were found in cRT, sRT PPT, Mini-Mental State Examination, Stroop test and digit span forward and backward. Significant differences were found in JHFT and RMT changes in the affected hemisphere ($p < 0.05$). The effects lasted 2 weeks.
(Kim et al., 2006)	1	**Group A:** Active iM1 10 Hz rTMS (160 pulses, 80% RMT) + Finger Motor Task **Group B:** Sham iM1 10 Hz rTMS (160 pulses, 80% RMT) + Finger Motor Task	-Movement accuracy and movement time. -MEP.	Group A showed significant differences in all outcome measures ($p < 0.05$).
(Takeuchi et al., 2005)	1	**Group A:** Active cM1 1 Hz rTMS (25 min, 90% RMT) **Group B:** Sham cM1 1 Hz rTMS (25 min) Motor training was done in both groups until one day before stimulation	-TCI duration, MEP and RMT. -Acceleration and Pinch Force.	The improvement of motor function (Acceleration and Pinch Force) after rTMS was significantly associated with the reduction of TCI duration in active rTMS group ($p < 0.05$).
(Mansur et al., 2005)	1	**Group A:** Active cM1 1 Hz rTMS (600 pulses, 100% RMT) **Group B:** Sham cM1 1 Hz rTMS (600 pulses, 100% RMT) **Group C:** Active premotor cortex 1 Hz rTMS (600 pulses, 100% RMT)	-sRT, cRT. -Purdue Pegboard Test. -Finger Tapping.	There were significant improvements in sRT, cRT and Purdue Pegboard Test respect group A ($p < 0.05$). There were improvements in group C, but no significant.

Acronyms: ARAT: Action Research Arm Test; BBT: Box and Blocks Test; cTBS: Continuous Theta Burst Stimulation; cM1: Contralateral Primary Motor Cortex; CRT: Choice Reaction Time; CSP: Cortical Silent Period; FAS: Functional Ambulation Scale; FIM: Functional Independence Measurement; FMA: Fugl Meyer Assessment; ICF/IF: Intracortical Facilitation; ICI: Intracortical Inhibition; IHI: Interhemispheric inhibition; iTBS: Intermittent Theta Burst Stimulation; iM1: Ipsilateral Primary Motor Cortex; ISP: Ipsilateral Silent Period; JHFT: Jebsen-Taylor Hand Function Test; LLFDI: Late-Life Function and Disability Index; MAL: Motor Activity Log; MAS: Modified Ashworth Scale; MEP: Motor Evoked Potential; MMSE: Mini-Mental State Examination; MRC: Medical Research Council; NHPT: Nine Hole Peg Test; NMES: neuromuscular electrical stimulation; NS: number of sessions; PPT: Purdue Pegboard Test; PT: Physical Therapy; Ref: references of the selected articles; RMT: Resting Motor Threshold; ROM: Range of Motion; rTMS: Repetitive Transcranial Magnetic Stimulation; SICI: Short Intracortical inhibition; SRT: Simple Reaction Time; SSQOL: Stroke Specific Quality of Life Scale; STEF: Simple Test For Evaluating Hand Function; TCI: Transcallosal inhibition; TEMPA: Test Evaluant la Performance des Membres supérieurs des Personnes âgées; V50: The stimulus intensity at which MEP amplitude is 50% of the MEPMAX and WMFT: Wolf Motor Function Test.

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unilateral application with rTMS. Two studies did not present a control group.

3.5. Outcome measures

All the studies focused their evaluation on arm/hand motor function through scales or instrumental evaluation. Besides, spasticity, cognition, and/or neurophysiological variables were also assessed. Evaluation metrics were highly heterogeneous between studies. All studies performed assessments before and after treatment.

Motor function evaluation was carried out using diverse metrics. We categorized them according to the constructs they assess. General upper limb motor function was assessed with the Fugl-Meyer Assessment (FMA) in 15 studies, the Wolf Motor Function Test (WMFT) in 9 studies and the Motor Activity Log (MAL) in 5 studies. Two studies assessed upper limb strength with the Medical Research Council (MRC) scale. Gross manual dexterity was assessed by the Box and Blocks Test (BBT) in 7 studies. Fine manual dexterity was assessed by the Nine Hole Peg Test (NHPT) in 12 studies, by the Action Research Arm Test (ARAT) in 7 studies, by the Purdue Pegboard Test (PPT) in 3 studies and by the Jebsen Taylor Hand Function Test (JHFT) in 3 studies.

Regarding spasticity, its presence and intensity were assessed only in 9 studies and always using the Modified Ashworth Scale (MAS). Finally, instrumental measures were performed by 5 studies that assessed handgrip strength with dynamometry, and 7 studies that assessed pinch strength with a pinchmeter.

Regarding neurophysiological measures to detect changes in cortical excitability, TMS based measures using different stimulation models and coils were utilized in 16 studies. Among the metrics used were interhemispheric inhibition (IHI), intracortical inhibition (ICI), short interval intracortical inhibition (SICI), intracortical facilitation (ICF), ipsilateral silent period (ISP), cortical silence period (CSP) and motor evoked potential (MEP).

3.6. Effectiveness of rTMS on upper limb motor sequelae

Comparison 1: active rTMS vs sham rTMS. Active rTMS groups presented significant improvements in FMA-UE, ARAT, STEF, NHPT, WMFT, MAL, BBT, JHFT, movement accuracy, movement time, movement acceleration, pinch force, and hand grip. Only 5 studies did not find significant differences between groups (Harvey et al., 2018; Rose et al., 2014; Talelli et al., 2012; Tretriluxana et al., 2013).

Comparison 2: rTMS vs conventional rehabilitation. rTMS groups presented significant improvements in FMA-UE, FMA-TT, WMFT, MAS, MAL, SIS, BBT, grip and pinch strength, Modified Barthel Index (MBI), Functional Independence Measure (FIM), FAS, MMSE and reaction time. Only one study did not find significant differences with respect to the control intervention (Kwon et al., 2014). Regarding the rest of the control groups, those who used rTMS without any other adjuvant therapy or audiotape, a tape-guided relaxation program with soothing music, found significant improvement in the experimental group according to FMA, WMFT, BBT and MBI.

Comparison 3: high-frequency rTMS vs low-frequency rTMS. Significant improvements in favor of the contralateral application with low-frequency stimulation, compared to the ipsilateral application with high-frequency, was found for FMA-UE, grip strength, MAS, NHPT, BBT, WMFT, and SIS.

Comparison 4: unilateral rTMS vs bilateral rTMS. Experimental groups presented significant improvements in favor of bilateral protocols not only in acceleration and pinch force (Takeuchi et al., 2009) but also in MRC, FMA-UE, WMFT, and reaction times (Sung et al., 2013)

Comparison 5: active TBS vs sham TBS. Stimulation with active TBS was significantly more effective than sham TBS according to FMA-UE, MAS, MAL, ARAT and SIS, except in one study (Talelli et al., 2012).

3.7. Methodological quality

The included studies had a mean PEDro score of 7.09 ± 1.97 (F.S.C) and 7.22 ± 1.80 (Y.G.Z) points. Figure 2 represents the distribution of total scores of each article according to both reviewers. Moderate agreement was found between raters according to the kappa value ($k = 0.61$). The values show good general methodological quality of the sample of included studies. Inclusion of a control group as well as double-blind and/or randomized designs correlated with higher PEDro scores.

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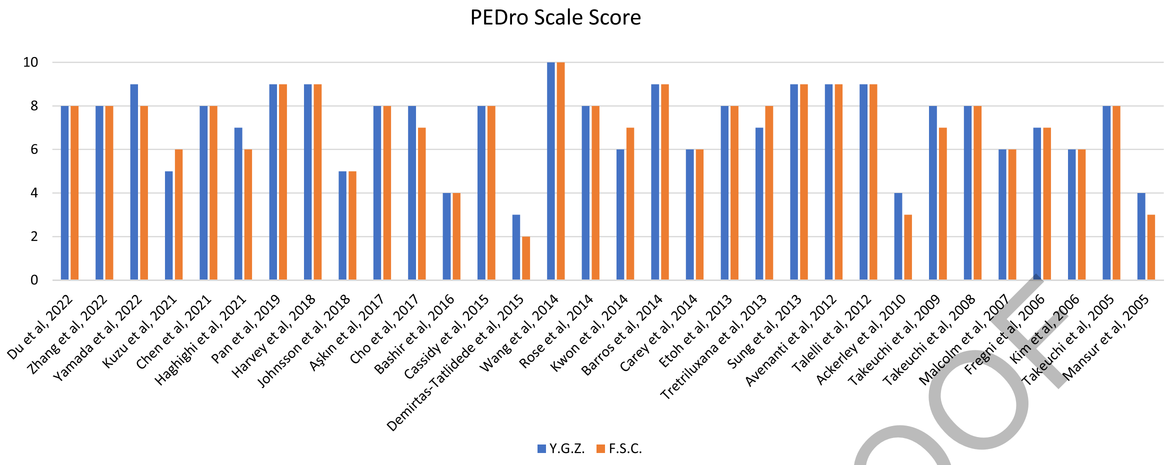

Fig. 2. Total PEDro score of the included studies assigned by each reviewer.

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4. Discussion

This review systematically analyzed the evidence from clinical trials published to date to determine the effectiveness of rTMS on upper limb motor function in subacute and chronic stages after stroke. The findings indicate that there is evidence that supports its use in clinical practice. In the variables included in our review, regardless of the time of treatment initiation, rTMS seems to have a positive effect on the functionality of the upper limb when evaluated with functional tests. This is consistent with literature data showing beneficial effects of rTMS in the acute phase of evolution (Chen et al., 2022).

Recent systematic reviews with meta-analyses (Chen et al., 2022) have proven the effectiveness of rTMS on general upper limb motor function measured with the FMA-EU. However, its effects on other motor function domains such as strength, dexterity or muscle tone have been previously overlooked. In this review, we synthesize evidence that suggests that rTMS might be effective on these constructs, which contribute to the performance of activities of daily living. Furthermore, we also synthesize evidence on the neurophysiological correlates of these functional improvements through the collection of cortical excitability measures.

Thirty-two studies matching our inclusion criteria were included in this review, published between 2005 and 2022. Most of them ($n = 19$) had good methodological quality according to PEDro score (≥ 7), which guarantees the validity of the conclusions extracted. However, there was large heterogeneity among the included studies regarding sample size, brain lesion topography and stroke type. Some studies had very limited sample sizes ($n = 3$) and most of them did not include more than 50 patients. Only one study included 240 individuals. Furthermore, there was consistent underreporting of sample size calculations, which may have introduced statistical biases. Although cortical and subcortical topography of the lesions and their size might determine the effect of the stimulation (Ameli et al., 2009), most of the studies made no difference between patients with different etiologies or topography of stroke. Nine studies only included people with ischemic stroke, but their results were not significantly different from those including hemorrhagic stroke.

Our review identified a greater number of low-frequency protocols applied over the contralesional hemisphere compared to interventions with high-frequency stimulation over the affected hemisphere (21 vs. 5 studies). Nonetheless, there is controversy on the relative effectiveness of these interventions and whether one is superior to the other in terms of their clinical effects on upper limb motor function. The present review is in accordance with previous meta-analyses suggesting more robust effects of low-frequency rTMS in comparison to high-frequency stimulation in chronic stroke (Chen et al., 2022), although these findings are far from definite. Moreover, there is emerging evidence that low-frequency stimulation over the unaffected hemisphere might concomitantly disrupt ipsilateral motor commands, and in consequence, might impair non-paretic upper limb function, deteriorating bimanual coordination and trunk control (Takeuchi & Izumi, 2012). Although these findings are preliminary and should be confirmed in large scale clinical trials, researchers and clinicians should be aware of the possible adverse effects of this type of rTMS. Much more high-quality research should be done to confirm these results and develop evidence-based recommendations which fairly consider the strengths and weaknesses of unilateral rTMS interventions.

There is currently contradictory evidence regarding the superiority of bilateral protocols combining low-frequency and high-frequency rTMS interventions in comparison to their isolated use. We found that bilateral stimulation was superior to unilateral rTMS, but recent meta-analyses did not (Chen et al., 2022). According to the studies included in our review, bilateral rTMS was more effective than unilateral stimulation in acceleration and pinch force, as well as MRC, FMA-UE, WMFT and reaction times (Takeuchi et al., 2009; Sung et al., 2013). There is therefore incipient evidence that bilateral protocols are also effective in improving some upper limb motor sequelae in the chronic stage after stroke. However, due to the small number of trials that undertook bilateral application, more research should be done on the study of its effects in comparison with unilateral protocols.

Besides safety guidelines (Kim & Paik, 2020), there are no consensus recommendations on the most effective dosing (intensity, number of pulses, or sessions) that should be used for stroke rehabilitation. This directly contrasts with rTMS applications for depression and chronic pain, which have recommendations based on a high level of evidence that inform about the site of stimulation and the type of frequency to use. However, it seems that the safety of rTMS interventions is consolidated, as none of the included studies (unilateral or bilateral) reported severe adverse effects like seizures, although it should be noted that safety assessment was not their primary aim.

With respect to the number of sessions, previous studies suggest that five sessions of rTMS have more beneficial effects on the function of the upper extremities compared to a single session or more than 10 sessions (Zhang et al., 2017). Our analysis showed that the most common protocol used 10 sessions (13 studies) distributed in two weeks. Notwithstanding, there is evidence suggesting that there are beneficial effects on functional and physiological outcome measures even when only one session was delivered (Ackerley et al., 2010; Bashir et al., 2016; Carey et al., 2014; Kim et al., 2006; Kwon et al., 2014; Mansur et al., 2005; Takeuchi et al., 2008; Tretriluxana et al., 2013). No security concerns were given to justify the low number of sessions. On the other hand, there is no certainty of the real duration of the effects of rTMS protocols in stroke as there is no long-term follow-up (> 3 months) or the effect of repetition of the protocol was evaluated.

Most people with stroke receive physical therapy as the basis of their rehabilitation. Thirteen studies included muscle activation or physical therapy alongside rTMS treatment and showed that better outcomes were observed when physical activation was performed during (Etoh et al., 2013) or after (Aşkın et al., 2017; Avenanti et al., 2012; Barros Galvão et al., 2014; Harvey et al., 2018; Kuzu et al., 2021; Rose et al., 2014; Takeuchi et al., 2008, 2009; Vongvaivanichakul et al., 2014; Wang et al., 2014; Yamada et al., 2022; Zhang et al., 2022) rTMS with limited effects when performed before neuromodulation (Avenanti et al., 2012). Nonetheless, one study (Zhang et al., 2022) used robot-assisted rehabilitation with and without iTBS priming showing more efficacy of the primed protocol and two studies tested successfully the effect of 6 Hz priming before 1 Hz inhibition (Carey et al., 2014; Cassidy et al., 2015). All these priming approaches may cause better outcomes due to the combination of different complementary neurophysiological mechanisms. This has led to justifying different combinations of non-invasive brain stimulation techniques (Lang et al., 2004) or pairing cortical neuromodulation with subsequent motor learning (Jung & Ziemann, 2009). In the case of physical activity priming, fatigue may be a factor inhibiting cortical excitability (Sharples et al., 2016) and thus impairing the proper activation by subsequent rTMS with poor clinical results when compared to rTMS priming.

The lack of homogeneity regarding outcome measures of upper limb motor recovery makes difficult to comprehensively compare between different rTMS protocols. Aside from the effect on general upper limb motor function measured with the FMA, our review also found positive effects on other specific domains that contribute to the overall function. MAS was used to evaluate spasticity in nine studies, although only five showed a positive effect (Aşkın et al., 2017; Barros Galvão et al., 2014; Chen et al., 2021; Du et al., 2022; Kuzu et al., 2021).

In fact, this review found that rTMS not only improves functional measures related to the affected upper limb, but generalized improvements were also evidenced (Aşkın et al., 2017; Avenanti et al., 2012; Barros Galvão et al., 2014; Fregni et al.,

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2006; Hirakawa et al., 2018; Vongvaivanichakul et al., 2014; Wang et al., 2014), as handgrip strength (Demirtas-Tatlidede et al., 2015), as well as acceleration and pinch force improvements (Takeuchi et al., 2008, 2009). Furthermore, some studies suggested that there may be an association between cortical neurophysiological variables (i.e. resting motor threshold reduction, changes in MEP amplitude) and improved function in the affected upper limb after stroke (Di Lazzaro et al., 2008; Yozbatiran et al., 2009). Nevertheless, neurophysiologic evaluation and cortical excitability measures were not related to clinical effects in most of the included studies nor the other way around. Only three studies demonstrated significant changes in neurophysiological metrics and significant clinical and functional improvements (Cassidy et al., 2015; Takeuchi et al., 2009; Avenanti et al., 2012). Although cortical excitability measures have been correlated with motor performance after stroke (Huynh et al., 2016), it seems that clinical improvement is not always related to measurable neurophysiological changes with the selected metrics, preventing from identifying causal relationships between them. This highlights that, at present, the underlying neural mechanisms of stroke recovery are still poorly understood and multiple hypotheses might provide insight into the neuroplastic changes mediating behavioral improvements observed after traditional rehabilitation and non-invasive neuromodulation interventions.

Although cognitive effects were not the main objective of any of the included studies, cognitive variables were included in six studies, and three of them presented significant improvements according to the MiniMental State Examination (MMSE), Simple Reaction Time (sRT) and Choice Reaction Time (cRT) (Aşkın et al., 2017; Bashir et al., 2016; Mansur et al., 2005). The three other studies did not reveal cognitive improvements (Carey et al., 2014; Fregni et al., 2006; Sung et al., 2013).

4.1. Limitations

Due to the variation in rTMS procedures (duration, intensity), outcome measures, control intervention protocols, and duration of follow-up, it was not possible to pool data for quantitative analyses, which is the most important limitation of this review. In addition, the associated costs and or economic benefit of rTMS were not reported in any study, as it was not their main aim. Therefore, there is currently a knowledge gap in terms of cost-effectiveness of rTMS interventions.

5. Conclusions

Based on the results of this systematic review, interventions using rTMS over M1 seem to be effective for improving upper limb motor function in people with subacute and chronic stroke. Different rTMS protocols, including inhibition of the contralesional hemisphere, facilitation of the lesioned hemisphere, and bilateral application through their combination, have proven to be effective to this purpose. Protocols combining conventional rehabilitation approaches with rTMS have been found to be more effective when the stimulation is delivered before motor training programs.

To capture meaningful information on the effectiveness of rTMS, the effects of neuromodulation treatments should be measured with combinations of both instrumental and clinical scales examining improvements on functional outcomes instead of tests measuring specific motor function constructs (e.g., muscle strength or acceleration). Neurophysiological measures are not always correlated with clinical effects. Studies considering minimal clinical differences and guidelines for maximum dosing should be elaborated to generalize the use of these protocols in clinical practice.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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Study Protocol

Clinical Effects of Immersive Multimodal BCI-VR Training after Bilateral Neuromodulation with rTMS on Upper Limb Motor Recovery after Stroke. A Study Protocol for a Randomized Controlled Trial

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Abstract: *Background and Objectives:* The motor sequelae after a stroke are frequently persistent and cause a high degree of disability. Cortical ischemic or hemorrhagic strokes affecting the cortico-spinal pathways are known to cause a reduction of cortical excitability in the lesioned area not only for the local connectivity impairment but also due to a contralateral hemisphere inhibitory action. Non-invasive brain stimulation using high frequency repetitive magnetic transcranial stimulation (rTMS) over the lesioned hemisphere and contralateral cortical inhibition using low-frequency rTMS have been shown to increase the excitability of the lesioned hemisphere. Mental representation techniques, neurofeedback, and virtual reality have also been shown to increase cortical excitability and complement conventional rehabilitation. *Materials and Methods:* We aim to carry out a single-blind, randomized, controlled trial aiming to study the efficacy of immersive multimodal Brain–Computer Interfacing-Virtual Reality (BCI-VR) training after bilateral neuromodulation with rTMS on upper limb motor recovery after subacute stroke (>3 months) compared to neuromodulation combined with conventional motor imagery tasks. This study will include 42 subjects in a randomized controlled trial design. The main expected outcomes are changes in the Motricity Index of the Arm (MI), dynamometry of the upper limb, score according to Fugl-Meyer for upper limb (FMA-UE), and changes in the Stroke Impact Scale (SIS). The evaluation will be carried out before the intervention, after each intervention and 15 days after the last session. *Conclusions:* This trial will show the additive value of VR immersive motor imagery as an adjuvant therapy combined with a known effective neuromodulation approach opening new perspectives for clinical rehabilitation protocols.

Keywords: stroke; repetitive transcranial magnetic stimulation; BCI-VR training; motor skills; upper limb

1. Introduction

Stroke is a leading cause of long-term disability; it reduces mobility in more than half of stroke survivors age 65 and over [1].

Despite the lack of objective prognostic factors regarding the patient's functionality after a stroke, we know that age, the level of initial disability, and the location and size of the lesion are elements that affect the evolution of post-stroke rehabilitation [2].

After a stroke, the recovery of lost functions in the brain is achieved thanks to reorganizing networks in a process known as plasticity. Some damaged brain tissues may recover, or undamaged areas may take over some functions.

One of the most relevant aspects of the rehabilitation prognosis is the time of evolution. After a stroke, improvement is noticeably reduced over the second month, finding stabilization around the sixth month. One of the reasons that explain this fact is the reduction of neuroplasticity [3]. There are indicative studies that reflect that, six months after a stroke, more than 60% of subjects will have a non-functional hand for Basic Activities of Daily Living (BADL), and 20–25% will not be able to walk without assistance [4]. These impairments determine the important global burden that stroke represents [5]. It is relevant to emphasize that the degree of disability after the rehabilitation process will be determined by the combination of existing motor, sensory, and neuropsychological deficiencies [6].

In the last years, several non-invasive neuromodulation techniques have been shown efficient to enhance plasticity and stroke recovery. Among these interventions, we can find exogenous neuromodulation, meaning that the neuromodulator stimulus comes from an external source, as is the case with rTMS (repetitive transcranial magnetic stimulation), which has the capacity to change the cortical excitability depending on the frequency of the magnetic pulses. Low frequencies ($\leq$1 Hz) reduce local neural activity, and high frequencies ($\geq$5 Hz) increase cortical excitability [7]. This technique has been successfully used bilaterally, stimulating the injured hemisphere and inhibiting the healthy one, to treat the interhemispheric inhibition phenomenon in stroke patients as it influences stroke recovery [8]. Although up to date there are some consensus recommendations about dosing and parameters of rTMS in some clinical applications such as pain [9] and depression [10], there is no clear consensus about the recommended parameters in stroke due to the heterogeneity of the currently published studies [11].

On the other hand, there are endogenous neuromodulation techniques that depend on the capacity of the subject to modulate its own brain activity. This can be achieved using neurofeedback (NFB), which consists of recording information of brain activity using electroencephalography (EEG) or functional magnetic resonance (fMRI) and displaying it to the subject in such a way that he can receive real-time information of his own brain function. Virtual reality allows a new dimension on the neurofeedback immersion and is likely to increase its efficacy [12]. Stroke patients have been trained to reinforce certain EEG rhythms related to motor performance using the NFB technique showing favourable effects on rehabilitation outcomes [13].

Some other techniques aiming to increase brain plasticity use the practice of imagination of movement of the affected hemibody [14]. This is known as motor imagery [15] and can also be enhanced through the use of brain–computer interfaces [16]. All the neuromodulation techniques are used to complement but not as a replacement of conventional rehabilitation [17,18].

On the one hand, exogenous neuromodulation effects are produced mainly by changes directly induced in cortical excitability [19], and on the other hand, endogenous neuromodulation is believed to have more widespread subcortical effects [20,21]. Currently, the exact mechanisms underlying motor recovery using neuromodulation techniques are still being investigated. Simultaneous activation of inputs and outputs to cortical motor areas is believed to trigger Hebbian plasticity, which strengthens cortical-subcortical connectivity. Improvements in this cortical-subcortical connectivity have been linked to better motor recovery after stroke [22,23].

One of the probable causes of the short-term effects of these techniques is the ceiling effect of changes in cortical excitability that can be achieved non-invasively, but despite the good results achieved with the use of non-invasive neuromodulation techniques individually, there is a shortage of validated neurorehabilitation protocols that integrate different approaches that have been proven to be effective individually.

It has been suggested rTMS may be a good conditioning method whose effect would be to increase brain plasticity that would improve the results of subsequent rehabilitation [24]. There are already several studies that compare rTMS alone with its combination with motor rehabilitation showing good results when rTMS is used as a primer that may help to reach a greater cortical activation and boosting plasticity [8,25–27]. Motor imagery and action observation have been shown to effectively activate the same brain areas as an actual movement [28]; up to date, it has not been combined with rTMS.

There is increasing evidence of Brain–Computer Interfacing (BCI) efficacy as rehabilitation technology in patients with severe motor impairments [29]. This is the case with NeuRow [30]. It is an immersive multimodal Brain–Computer Interfacing (BCI) training paradigm that combines motor imagery and embodied neurofeedback using virtual reality. NeuRow has been designed to be used by the chronic stroke patient population [31], and its efficacy has been shown in a pilot study. It shows clear improvements and recovery regarding motor function in terms of clinical scales (FMA, MAS, SIS), self-reported scales, electrophysiological data, and brain imaging data (fMRI). There is evidence that BCI-based rehabilitation promotes lasting improvements in motor function in chronic stroke patients with hemiparesis [32].

Both approaches, the NeuRow training paradigm (NeuroRehabLab, Funchal, Portugal) and bilateral rTMS protocols, are likely to complement their effects, achieving a stronger neuroplasticity enhancement in stroke patients. Both have been used separately for the treatment of motor sequelae in the upper limbs after stroke [8,31,33]. The effects of these combined techniques are not likely to be based only on the increase of cortical excitability but also on subcortical mechanisms.

The efficacy of rTMS and motor imagery has been previously demonstrated, but their combination has not been tested yet. The main objective of this study is to carry out a double-blind, randomized, controlled trial aiming to study the clinical effect of immersive multimodal BCI-VR training using NeuRow system (NeuroRehabLab, Funchal, Portugal) after bilateral neuromodulation with rTMS compared to bilateral rTMS plus conventional rehabilitation in upper limb motor sequelae after subacute stroke (>3 months). We will look for changes in: 1. isometric strength in the upper limb; 2. functional motor scales of the upper limb; 3. hand dexterity; and 4. cortical excitability changes. Our main hypothesis is that multimodal BCI-VR training will be superior to the use of conventional motor imagery as adjuvant therapy to bilateral rTMS.

This protocol combines techniques that have proven to be cost-effective [34]. If it is shown that the clinical improvement with this combination is significant, it will open a new line of combined neuromodulation approaches to reach an effective method for the upper limb motor neurorehabilitation after a stroke.

2. Materials and Methods

The SPIRIT 2013 Checklist has been used to assure the quality of the protocol [35]. This protocol has been registered in trials.gov with the number NCT04815486.

2.1. Study Design and Participants

Participants will be recruited in the Brain Injury Unit or Rehabilitation Unit of the Beata María Ana Hospital; patients referred from other centres and self-referred patients will also be considered. The subjects included will be assessed by a neurologist (JPR) and a physiotherapist (FJS). All patients will have had a hemispheric ischemic or hemorrhagic stroke (>3 months after stroke) diagnosed in at least one brain-imaging test and presenting motor sequelae in the upper limb. We have ruled out the first three months since the phenomenon is known as "spontaneous recovery" [36] takes place in time, and therefore, it would be difficult to discern the cause of the clinical improvement that we expect from patients. The patients included in the study will have an alteration of mobility and/or functionality in the upper limb related to the stroke and with a score equal to or greater than 25 according to the Fugl-Meyer Assessment for upper extremity (FMA-UE) [37]. The rest of the specific inclusion and exclusion criteria are shown in Table 1.

Table 1. Inclusion and exclusion criteria.

INCLUSION CRITERIA	EXCLUSION CRITERIA
Older than 18 years old.	History of seizure or brain aneurysm
Ischemic or hemorrhagic cerebrovascular injury diagnosed by a neurologist and who have at least one brain-imaging test	Pacemakers, medication pumps, metal implants in the head (except dental implants)
Onset of hemispheric ischemic or hemorrhagic stroke >3 months	Clinical instability
Kinesthetic and Visual Imagery Questionnaire (KVIQ) >55.	Muscle tone in the wrist and hand with a modified Ashworth scale (MAS) score equal to or higher than 3 in the wrist
Stability in antispastic medication for more than 5 days	Other pre-existing neurological diseases or previous cerebrovascular accidents with sequelae
Able to read and write	Aphasia
Sufficient cognitive ability to understand and perform tasks: Token Test >11	Previous TMS after stroke Hemispatial neglect (Bells Test >6 omissions on one side) Visual problems
	Flaccid paralysis Brunnstrom's stage = 1

The study design corresponds to a randomized, single-blind, controlled clinical trial in which patients are randomly assigned to two groups: 1. Conventional rehabilitation + bilateral rTMS + Immersive multimodal BCI-VR training system NeuRow (NeuroRehabLab, Funchal, Portugal), and 2. Conventional rehabilitation + bilateral rTMS.

Regarding adherence strategies, sessions missed up to the established limit will be restored the following week. Flexible therapy schedules will also be offered, and patients' families will be contacted directly by phone to confirm evaluation dates, thus reinforcing treatment adherence [38].

To calculate the sample size, the GRANMO calculator was used *(Sample size and power calculator (v. 7.12.), Institut Municipal d'Investigació Mèdica, Barcelona, Spain)*. Accepting an alpha risk of 0.005 and a beta risk of 0.2 in a one-sided contrast, 23 subjects are required to detect a difference equal to 4.9 points, the minimal clinically important difference of the Fugl-Meyer assessment scores for wrist/hand [39]. Considering a 15% loss, it will be necessary to reach a sample of 21 patients in each group.

Randomization and blinding will be made using the application Research Randomizer (Social Psychology Network, Middletown, CT, USA) [40] to form the groups. A code consisting of two digits (1 and 2) will be used. The application offers a random list of 30 numbers with digits 1 and 2. This sequence will be carried out remotely by a blind researcher who is not involved in other investigation procedures. After the randomization process, another blind staff member will assign patients among groups. The allocation concealment will be made with closed, sealed, and sequentially numbered envelopes. The evaluators will receive the patients in a different room, ignoring which group they belong to.

To assess the adverse effects, participants will be asked at the end of each session if they experienced effects such as tingling, burning, headache, and drowsiness, among others and the intensity of this sensation. Guidelines for safety on rTMS protocols will be followed [41]. However, no severe adverse effects are expected. Any adverse effect will be notified to a licensed medical doctor. Management of possible adverse effects will be individualized and according to the severity.

Regarding data processing security, data will be recorded separately and will be anonymized and guarded following current European data protection laws. All data will be recorded and verified twice in a database specifically designed for the studies.

2.2. Intervention Protocol

The two intervention protocols have been extracted from previous publications of successful application on upper limb rehabilitation. On the one hand, the investigation by Takeuchi et al. showed significant improvements in those subjects who received bilateral rTMS stimulation (based on the principle of interhemispheric inhibition) [8]. On the other hand, Vourvopoulos et al. published a pilot study using NeuRow, showing a high performance [33].

The intervention will consist of the two therapies administered sequentially on the same day rTMS application before the immersive multimodal BCI-VR training, or just rTMS application, in the control group. The sequence of administration has been decided based on preliminary results of the NeuroMOD project (unpublished) from our group, revealing that patients receiving rTMS prior to NFB had a better performance.

2.2.1. rTMS Stimulation

Firstly, single-pulse TMS will be performed to acquire a resting motor threshold (rMT). Magstim Rapid2 (Magstim Company, Whitland, Wales, UK) device with an air-cooled 70 mm figure-of-eight magnetic stimulator coil will be used. For all assessments, the magnetic stimulator coil will be placed on M1 in the assessed hemisphere, and a surface electromyogram from the contralateral first dorsal interosseous muscle will be recorded using surface EMG using CED Signal Software (CED, Cambridge, UK). EMG recordings will be recorded and stored in a computer for offline analysis. Stimulation output intensity used to reach the resting motor threshold (RMT) will be used to calculate the parameters for the stimulation sessions.

rTMS parameters will be as described by Takeuchi et al. [8]: 90% of the RMT at 10 Hz, 1000 pulses, 5 s intertrain interval on M1 of the lesioned hemisphere and after 5 min rest period, the contralateral M1 area will be stimulated with 90% of the RMT at 1 Hz, 1000 pulses, with a 50 s intertrain interval. This will be performed in 10 consecutive daily sessions (Monday to Friday).

2.2.2. Immersive Multimodal BCI-VR Training

NeuRow is a gamified BCI training paradigm in VR that allows patients to perform similar motor actions as they would do in real life. NeuRow is rendered through a head-mounted VR headset with 90° horizontal field-of-view and haptic feedback delivered through 2 controllers in both hands.

The paretic limb should be positioned in a resting position on the table. Before setting up the EEG cap plus VR headset in each session, a previous training will be carried out with the following instructions:

1. Ask the patient to perform the rowing movement with both upper limbs with external facilitation of the paretic side.
2. Ask the patient to imagine the movement with eyes closed, focusing on his internal perspective and on the sensation of rotation. Imagine the hand closed in a fist and feel the arm weight and contraction of arm muscles.
3. Imagine the movement slowly and increase their speed.
4. The best strategies will be identified for each participant. The patient reports in detail what he felt/tried to visualize; the researcher will give feedback and will also give a description of the sensation of the movement to the participant during the motor imagination, describing the sequence of movements required for rowing (elbow stretched, closed hand grasping the paddle, etc.).

5. The patient will be asked if he succeeds in imagining the tasks.

It is very important that the movement is natural and biomechanically correct. This training will be carried out daily and prior to the application of the NeuRow system.

Before the actual VR training, the acquisition of the EEG will be carried out. An experienced technician will acquire EEG through a BCIs system with 64 active electrodes equipped with a low-noise biosignal amplifier and a 24 bit A/D converter at 256 Hz (BrainVision actiCHamp biosignal amplifier, Brain Products GmbH, Gilching, Germany). The spatial distribution of the electrodes will cover primarily the motor and somatosensory areas of the brain. Specifically, the Frontal (F3, Fz, F4), Frontal-Central (FC5, FC6), Central (C3, Cz, C4), Central-Parietal (CP5, CP1, CP2, CP6), and Parietal (P3, Pz, P4) in a small Laplacian configuration for spatial filtering (Figure 1). EEG data acquisition and processing will be performed through the OpenVibe platform [42], which will transmit the data via the Lab Streaming Layer (LSL) protocol to control the virtual environment.

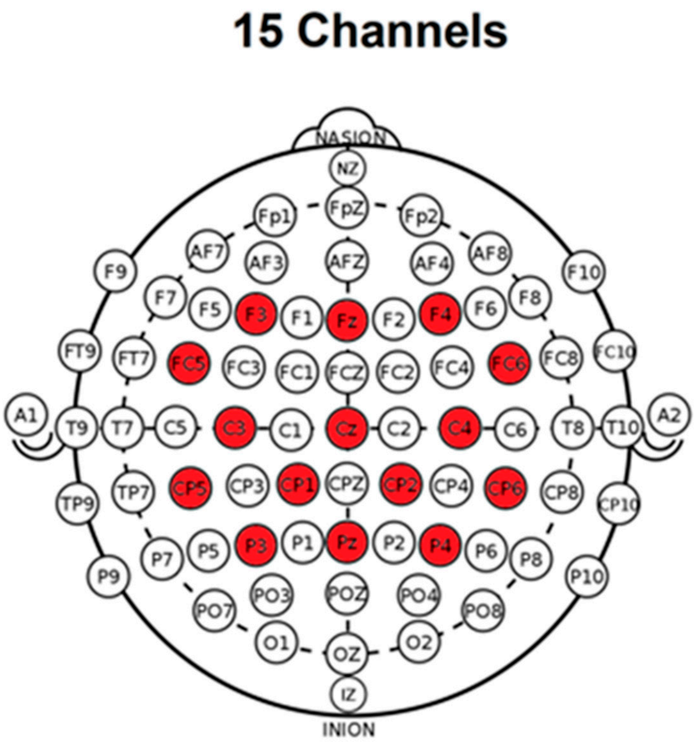

Figure 1. The spatial distribution of the electrodes. In red, the active electrodes.

Secondly, the BCIs training protocol designed and adapted based on the Graz-BCI paradigm will be used [43]. The first step will be the acquisition of the raw EEG data to extract features to train a classifier to distinguish Right and Left imagined hand movements. Thus, the patient will have to perform mental imagery of the corresponding hand according to the presented stimuli on the screen. The training session will be configured to acquire data in 24 blocks per class (Right-or Left-hand imagery) in a randomized order. Afterwards, the data will be filtered both spatially and temporarily between the Alpha and Beta bands (8–30 Hz) for creating the feature vector.

During the training session, patients will wear a VR headset and see a boat and two high fidelity virtual arms gripping two oars in the first-person view; they will have to imagine the movement of each corresponding hand to rotate each oar and progress, observing the movement imagined on screen [31]. The game interface includes timekeeping and scoring. The goal of the task is to perform as many correct Motor Imagery sequences as possible in a fixed amount of time. To improve adherence, points will be awarded depending on the performance in each session [33,34]. The Motor Imaging training mode will be used. Training sessions will be performed in 12 non-consecutive sessions (Monday, Wednesday and Friday, during four weeks) lasting 30 min each, divided into 3 series of 7 min including initial training and break time to prevent fatigue [31].

2.3. Outcomes Measurement

Three evaluations will be carried out for each patient. A pre-intervention initial evaluation, a second evaluation the week following the end of the rTMS intervention, and a final evaluation after two weeks, when the NeuRow training finish. The first and third evaluation sessions will have an average duration of 120 min, and the second evaluation will have a duration of 60 min (Figure 2).

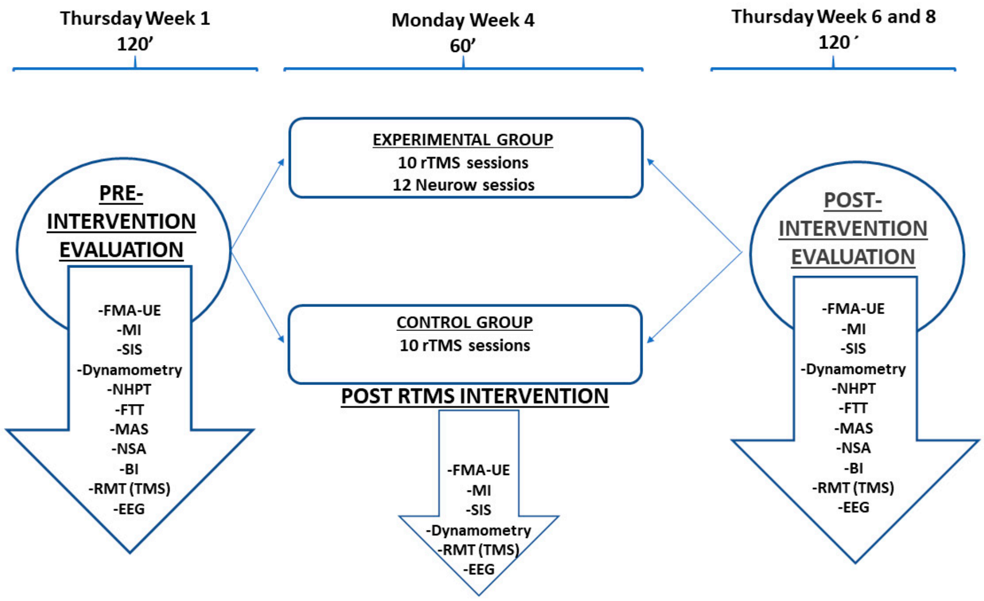

Figure 2. Schematic representation of intervention protocol. It includes the days of the week and duration of each block of outcomes measurement in pre- and post-evaluation (FMA-UE—Fugl-Meyer Assessment for upper extremity; MI—Motricity Index; SIS—Stroke Impact Scale; RMT—Resting Motor Threshold; NHPT—Nine Hole Peg Test; FTT—Finger Tapping Task; MAS—Modified Asworth Scale; NSA—Nottingham Sensory Assessment; BI—Barthel Index; EEG—Electroencephalogram. Thursday/90–120 min; Monday/60 min; Control Intervention/Monday to Friday/10 rTMS sessions and Experimental Intervention: Monday to Friday/10 rTMS sessions and Monday, Wednesday, and Friday/12 NeuRow sessions).

2.3.1. Main Outcomes

Due to the bilateral stimulation protocol used, all outcomes will be assessed on both sides.

Motricity Index of the Arm (MI): The upper limb section of the MI assesses muscle strength in 3 muscle groups, including grip, elbow flexion, and shoulder separation. Each movement is scored discreetly (0 if there is no movement, 9 if the movement is palpable, 14 if the movement is visible, 19 if the movement is against gravity, 25 if the movement is against resistance, and 33 if the movement is normal), obtaining a total score for the upper limb that ranges from 0 (severely affected) to 100 (normal). This assessment methodology has been widely used in rehabilitation progress evaluation [44,45] and counts with a normalized and weighted scoring system.

Dynamometry: A handheld analogic dynamometer (Jamar® Plus+ Hand Dynamometer, 0–90 kg) (Performance Health Supply, Nottinghamshire, UK) will be used to assess isometric grip strength. Patients will be positioned in a straight back chair with both feet on the floor and the forearm resting on a stable surface. Patients will perform a maximal isometric grip contraction until they reach maximal force output. Three measures will be taken with 1 min rest between tests, and the mean value will be recorded. This provides an objective evaluation of handgrip strength that will allow pre- and post-protocol comparison.

Fugl-Meyer Assessment for upper extremity (FMA-UE): The FMA-UE is an observational rating scale that assesses sensorimotor impairments in post-stroke patients. It also includes four subscales: A. Upper Extremity (0–36), B. Wrist (0–10), C. Hand (0–14), and D. Coordination/Speed (0–6), composing a total maximum score of 66 points. This scale is considered adequate to detect changes in motor recovery in patients who have suffered a stroke because of its wide evaluation items [46]. The minimum clinically important difference (MCID) for the upper limbs has been determined and ranges from 4.25 to 7.25 points, depending on the evaluated region of the upper limb [47].

Stroke Impact Scale (SIS): The SIS is a stroke-specific quality of life instrument to assess the quality-of-life impairment after stroke only considering the physical domain. It presents 4 subscales, but only the hand function domain will be evaluated. The SIS is a brief and easy instrument that considers specifically motor impairment impact over daily living activities [37].

2.3.2. Secondary Outcomes

Computerized Finger Tapping Task (FTT): The FTT measures motor function and is very sensitive to the slowing down of responses. In this task, following the Strauss application norms, the participants will be instructed to sit comfortably in front of a computer and press the spacebar on the keyboard as fast as possible and repeatedly with the index finger. Five 10 s attempts will be performed with each hand. The average time between two consecutive taps in the five trials will be the dependent variable.

Nine Hole Peg Test (NHPT): The NHPT evaluate the impairment in upper limb dexterity [48]. Patients must pick up as quickly as possible nine pegs from a container one by one unimanually and transfer them into a target pegboard with nine holes until filled. Then, they must return them unimanually to the container. The outcome variable will be the time spent to complete the whole task [49]. The NHPT is considered reliable [50], valid, and sensitive to change [51] among stroke patients.

Modified Ashworth Scale (MAS): The MAS is one of the most used tools for the assessment of spasticity [52]. Patients will be in the supine position with their arms by their side and with their head in a neutral position [53]. The MAS is markedly responsive in detecting the changes in muscle tone in patients with stroke [54]. This scale will be used because secondary changes in spasticity are possible if motor changes are produced.

Nottingham Sensory Assessment (NSA): Somatosensory impairment of the upper limb occurs in approximately 50% of adults after stroke, associated with loss of hand motor function, activity, and participation. The measurement of sensory impairment in the upper limb is a component of rehabilitation that contributes to the selection of sensorimotor techniques that optimize recovery and provide a prognostic estimate of the function of the affected upper limb [55]. There are studies documenting changes produced in the sensation of the upper limb after the application of neurofeedback [56] and even after the intervention with motor imagery [57]. Since the protocol presents an intervention with the application of these techniques, it is possible that there will be changes related to the sensitivity after the use of the platform, NeuRow system (NeuroRehabLab, Funchal, Portugal) [31].

Barthel Index (BI): Accurately assessing the ADLs of stroke patients greatly helps in evaluating the efficacy of stroke treatments [58]. The Barthel Index was originally established to assess ADL in stroke patients and has been used extensively for this purpose [59].

Neurophysiological measurements of cortical plasticity changes:

TMS Resting Motor Threshold (RMT) in the first dorsal interosseous muscle or the abductor pollicis brevis muscle will be recorded to determine the cortical excitability changes and correlate them with the clinical outcomes.

EEG: Different measures of quantitative EEG will be collected. They have been shown to be very useful in evaluating stroke patients' recovery [60].

2.4. Data Analysis

Parametric tests will be used for the analysis of the results if compliance with the assumptions (normality and equality of variance) and the sample size allow it. These analyses include a Student's *t*-test for independent or paired samples, ANOVA of one or two factors and repeated measures, and Pearson correlations.

Due to the data nature, analyses will be completed with nonparametric tests such as $\chi 2$ and Wilcoxon. The residual effect, period effect, and sequence effect checks will be made.

In all analyses, a confidence level of 0.95 will be adopted. The data analysis will be carried out with the help of the statistical program SPSS 25.0 (SPSS Inc., Chicago, IL, USA).

2.5. Dissemination Plans

All results will be published in specialized scientific journals. Results will be made public through the social media of our institution.

3. Discussion

Non-invasive brain stimulation (NIBS) by repetitively activating circumscribed brain regions with magnetic stimulation has a promising future as an augmentative therapeutic approach to traditional physical therapy after stroke [61]. rTMS protocols based on interhemispheric inhibition compensation have been reported to compensate for this phenomenon and consolidate neuroplastic changes [62].

On the other hand, the results from the studies by Vourvopoulos et al. [31] and Ramos-Murguialday et al. [32], using multimodal immersive BCI-VR training, have also shown to improve the carry-over effect of the rehabilitation process evidenced in clinical scales, self-reported scales, electrophysiological data, and brain imaging data.

Several previous studies combining two different non-invasive neuromodulation approaches have been successfully tested in stroke patients' rehabilitation [63–65]. This is the first time that multimodal immersive BCI-VR training is evaluated as an enhancer of the proven efficacy or rTMS bilateral protocols focused on interhemispheric inhibition compensation.

It seems that in the rehabilitation of the hand after a stroke, the cortico-subcortical connectivity mechanisms are relevant [23]. Exogenous source neuromodulation (rTMS) constitutes a cortical excitability input that activates the cortex and cortico-spinal pathway in a top-down mechanism. On the other hand, endogenous neuromodulation (motor imagery) may be similar to bottom-up rehabilitation mechanisms elicited by physical motor activation activating cortical and subcortical mechanisms [66]. In this way, we think that by combining both therapies, we will further enhance the cortico-subcortical connectivity, and therefore, the clinical effects regarding the motor recovery of the affected limb will be greater.

The combination of these techniques with the extensive, objective evaluation of upper limb outcome will generate a new hypothesis about how the combination of different neuromodulation approaches affect homeostatic plasticity and, thus, motor recovery.

The validation of this protocol will determine the clinical utility of the combination of two non-invasive neuromodulation approaches to enhance the effect of conventional rehabilitation on stroke. The outcomes of this study will contribute to identifying if multimodal immersive BCI-VR training enhances the known effects of rTMS over interhemispheric inhibition. Neurophysiological and clinical prognostic factors of response to this protocol will be determined.

4. Conclusions

This trial will show the additive value of VR immersive motor imagery as an adjuvant therapy combined with a known effective neuromodulation approach opening new perspectives for clinical rehabilitation protocols. This therapy could potentially reduce the time required for hand rehabilitation or improve functional outcomes reducing long-term disability.

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Data Availability Statement: The data presented in this study will be available on request from the corresponding author.

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ORIGINAL REPORT

EFFECTS OF MOTOR IMAGERY-BASED NEUROFEEDBACK TRAINING AFTER BILATERAL REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION ON POST-STROKE UPPER LIMB MOTOR FUNCTION: AN EXPLORATORY CROSSOVER CLINICAL TRIAL

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Objective: **To examine the clinical effects of combining motor imagery-based neurofeedback training with bilateral repetitive transcranial magnetic stimulation for upper limb motor function in subacute and chronic stroke.**

Design: **Clinical trial following an AB/BA crossover design with counterbalanced assignment.**

Subjects: **Twenty individuals with subacute (*n* = 4) or chronic stroke (*n* = 16).**

Methods: **Ten consecutive sessions of bilateral repetitive transcranial magnetic stimulation alone (therapy A) were compared vs a combination of10 consecutive sessions of bilateral repetitive transcranial magnetic stimulation with 12 non-consecutive sessions of motor imagery-based neurofeedback training (therapy B). Patients received both therapies (1-month washout period), in sequence AB or BA. Participants were assessed before and after each therapy and at 15-days follow-up, using the Fugl-Meyer Assessment-upper limb, hand-grip strength, and the Nottingham Sensory Assessment as primary outcome measures.**

Results: **Both therapies resulted in improved functionality and sensory function. Therapy B consistently exhibited superior effects compared with therapy A, according to Fugl-Meyer Assessment and tactile and kinaesthetic sensory function across multiple time-points, irrespective of treatment sequence. No statistically significant differences between therapies were found for hand-grip strength.**

Conclusion: **Following subacute and chronic stroke, integrating bilateral repetitive transcranial magnetic stimulation and motor imagery-based neurofeedback training has the potential to enhance functional performance compared with using bilateral repetitive transcranial magnetic stimulation alone in upper limb recovery.**

Key words: repetitive transcranial magnetic stimulation; rTMS; motor imagery; neurofeedback; stroke; motor cortex; upper limb.

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LAY ABSTRACT

This study evaluated how a combination of 2 non-invasive neuromodulation treatments can help improve arm movement in individuals who have had a stroke. The treatments used were repetitive transcranial magnetic stimulation and mental practice through neurofeedback. Twenty patients participated and were assigned to 1 of 2 groups: either receiving 10 sessions of bilateral repetitive transcranial magnetic stimulation alone, or followed by an additional 12 sessions of neurofeedback during which participants imagined moving the affected arm. The results showed that both groups improved arm function and sensory function. However, the combination of repetitive transcranial magnetic stimulation and mental practice had better results than repetitive transcranial magnetic stimulation alone. Participants experienced a significant improvement in their ability to move and feel with the arm. The effects persisted at least 1 month after completing the treatment. This study shows the potential benefit of using combined neuromodulation therapies in subacute and chronic stages of stroke sequelae.

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Globally, an estimated 15 million people experience a stroke each year, many of who exhibit upper limb (UL) motor function deficits within 4 weeks after stroke (1, 2). One semester after the accident, more than 60% of subjects are unable to perform basic activities of daily living (ADLs) due to hand motor deficits (3). Among the primary sensory-motor deficits of the affected UL, loss of handgrip strength, alterations in muscle tone and motor control, and decreased superficial and deep sensitivity significantly impair independence (4).

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Conventional UL rehabilitation effectiveness is limited after 3–6 months following acute onset (5). This may be explained by a plateauing of the regain of function during this period; it is likely that the first 3 months correspond to the highest period of circuit plasticity in humans (6).

Repetitive transcranial magnetic stimulation (rTMS) is an exogenous neuromodulation technique that involves applying magnetic stimulation pulses to specific areas of the brain with the purpose of influencing cortical excitability and thus brain plasticity (7). Depending on the frequency of stimulation, this may have an excitatory or inhibitory effect (e.g. frequencies ≤1 Hz have an inhibitory effect, while frequencies ≥5 Hz increase excitability).

Conversely, neuromodulation can also be performed endogenously. One of the techniques widely validated for this is neurofeedback (NFB), a non-invasive technique that involves real-time monitoring and training of brain activity to improve self-regulation, through providing individuals with real-time information about their brainwave patterns, typically obtained through an electroencephalogram (EEG). This technique is often coupled with motor imagery (MI), providing real-time information about the brain's activity when imagining motor actions (8).

Both approaches have been used in stroke rehabilitation: rTMS appears to recover strength, increase manual dexterity, and UL functionality, and NFB and MI have been identified as effective strategies for improving motor rehabilitation (9–11).

These neuromodulation techniques have certain limitations, including the potential for short-lived results and significant variability in treatment response among individuals (12). In addition, the majority of efficacy studies conducted on these therapies have utilized small sample sizes, which hinders the generalizability of the results (13, 14).

These techniques are often combined with conventional rehabilitation; however, neuromodulation techniques are not commonly combined with each other. Previous evidence has indicated differences in the scope of neuromodulatory effects between endogenous modulation through MI-based NFB and exogenous modulation via rTMS, with MI-based NFB potentially showing a wider influence on subcortical regions, while rTMS tends to exert its primary impact on cortical areas (15). Therefore, both approaches may have complementary neurophysiological mechanisms to achieve more significant and persistent effects. However, their combination is largely unexamined.

The primary objective of this study was to investigate the clinical effects of adding MI-based NFB training to bilateral rTMS in individuals with stroke. The study hypothesis was that the clinical effects achieved with NFB training after bilateral rTMS would be superior to those of bilateral rTMS alone.

METHODS

Study design

A clinical trial following an AB/BA crossover design was conducted following the Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines (16). The protocol was prospectively registered in clinicaltrials.gov (unique identifier NCT04815486). The study was approved by an independent Clinical Research Ethics Committee at Hospital Universitario de Fuenlabrada, Madrid, following the principles of the Declaration of Helsinki 1964, updated 2013. All participants gave written informed consent before participating. None of the evaluators, therapists, or patients were blinded.

Participants

Sample size. Twenty consecutive participants fitting the eligibility criteria (see below) completed the interventions assigned. Fig. 1 shows the participant flow diagram. Four participants in the subacute stage (3–6 months) and 16 participants in the chronic stage (>6 months) of stroke were mostly recruited from the Brain Injury Unit or Rehabilitation Unit of Hospital Beata María Ana Madrid, Spain. Participants referred from other centres and self-referred patients were also included.

To determine the sample size in this study, various factors were considered. A previous study that utilized rTMS in the rehabilitation of motor sequelae in the UL of patients with chronic stroke has shown an effect size on Fugl-Meyer Assessment (FMA-UL) of Cohen's d = 1.31 (17). However, that effect size would not be expected in the current study because of: (*i*) the cross-over design, compared with the parallel-group design of the cited study; and (*ii*) the effect of NFB and the rTMS would not be expected to be as large as the previous ones, after accounting for the correlation between repeated measures in the same subjects, and because of the potential ceiling effect reached with the rTMS intervention alone. Therefore, an effect size of Cohen's d = 0.8 was chosen, considering that a large effect size was expected according to standard interpretation (18), but significantly smaller than that of previous studies, and therefore more conservative. Therefore, an effect size of Cohen's d = 0.8 was chosen, considering that a large effect size was expected according to standard interpretation (18), but that the expected effect size was significantly smaller than that of previous studies, and therefore more conservative. This calculation was performed in G*Power version 3.1 (Aichach, Germany /www.g-power.de). Accounting for an estimated loss rate of 20%, it was concluded that 23 subjects were needed.

Eligibility criteria. The eligibility criteria were as follows. Inclusion criteria: (*i*) age > 18 years, (*ii*) ischaemic or haemorrhagic cerebrovascular injury diagnosed by a neurologist and who have at least 1 brain-imaging test, (*iii*) onset of symptoms > 3 months, (*iv*) presence of upper limb motor sequelae due to stroke; (*v*) stability in anti-spastic medication for more than 5 days, (*vi*) able to read and write, and (vii) sufficient cognitive ability to understand and perform tasks (Token test > 11) (19). Exclusion criteria: (*i*) history of seizure or brain aneurysm, (*ii*) pacemakers, medication pumps, metal implants in the head (except dental implants), (*iii*) clinical instability, (*iv*) aphasia, (*v*) other pre-existing neurological diseases or previous cerebrovascular accidents with sequelae, (*vi*) previous rTMS interventions

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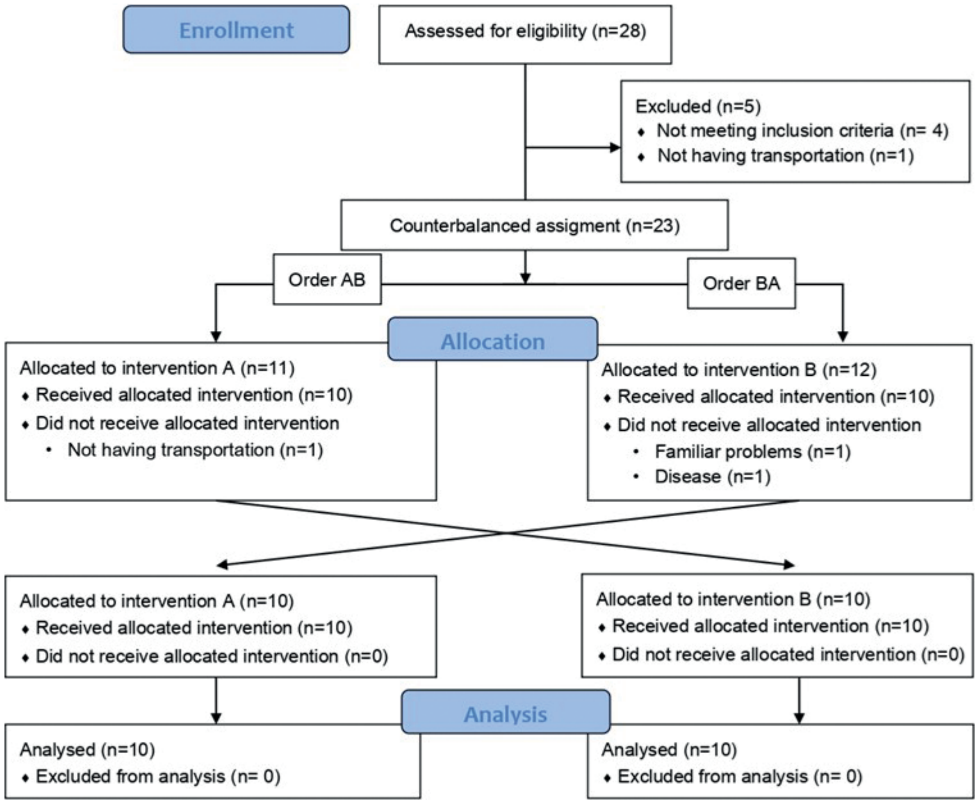

Fig. 1. Participant flow diagram according to Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines.

received after stroke, (*vii*) hemispatial neglect, and (*viii*) flaccid paralysis (Brunnstrom's stage <1) (20).

Interventions

Bilateral rTMS. A bilateral rTMS protocol was applied based on the approach of Takeuchi et al. 2009 (21). First, single-pulse TMS was delivered to obtain the resting motor threshold (RMT) using a Magstim Rapid2 device (Magstim Co., Whitland, UK) with a 70-mm figure-of-8 coil. The coil was placed on M1 contralateral to the stroke lesion, and in its homologous on the ipsilesional side as the hot spot was not always identifiable on the lesioned side. RMT was calculated using standardized methods published elsewhere (22).

The rTMS intervention protocol consisted of 10 consecutive daily sessions of bilateral stimulation over a period of 2 weeks (Monday to Friday). Each session comprised stimulation over both M1, starting with low-frequency (1 Hz) stimulation over the contralesional side and, after a resting period of 5 min, high-frequency (10 Hz) stimulation over the lesioned side. Each side received, at 90%RMT (or a maximum intensity of 52% of the default Magstim Rapid2 device maximum output according to its security system), a total of 1000 pulses divided into 20 trains of 50 pulses each, with a 5-s intertrain interval (21).

To assess side-effects, participants were asked at the end of each session whether they experienced tingling, headache or neck pain, drowsiness, and the intensity of these sensations. Safety guidelines for rTMS protocols were followed (23).

Motor imagery-based neurofeedback. NeuRow is a MI-based neurofeedback (NFB) training paradigm that allows patients to perform UL motor actions such as they would do in real life. It incorporates a brain-computer interface (BCI) system, which is based on MI and is guided by NFB with EEG.

NeuRow is rendered through a head-mounted virtual reality (VR) headset with a 90° horizontal field of view, and haptic feedback is delivered via 2 controllers in both hands. The paretic limb should be placed in a resting position on the table. After ensuring that the position of the patient and the UL were correct, the EEG cap and the VR viewer were configured. The task consisted of imagining performing unilateral rowing movements with each UL alternately. In the virtual environment, patients saw a boat and 2 high-resolution virtual arms grasping 2 oars in first-person view. The patient had to imagine the movement of each corresponding hand to turn each oar and move forward, observing the imagined movement on the screen. The goal of the task was to perform as many correct motor image sequences as possible in a fixed period.

EEG acquisition was performed using a BCI system with 64 active electrodes equipped with a low noise biosignal amplifier and a 256-Hz 24-bit A/D converter (BrainVision actiCHamp biosignal amplifier, Brain Products GmbH, Gilching, Germany). EEG data were acquired following the international placement of the 10–20 system as follows. The 15 electrodes for BCI were spatially distributed covering mainly the motor and somatosensory areas of the brain. Specifically, Frontal (F3, Fz, F4), Frontal-Central (FC5, FC6), Central (C3, Cz, C4), Central-Parietal (CP5, CP1, CP2, CP6) and Parietal (P3, Pz, P4) electrodes, in a small Laplacian configuration for spatial filtering, were used. Both the processing and the acquisition of the EEG data were carried out with the OpenVibe platform (24), which transmits the data through a Lab Streaming Layer protocol to control the virtual environment.

A BCI training protocol designed and adapted based on the Graz-BCI paradigm (25) was used. First, acquisition of raw EEG data was performed. Features were extracted in order to train a classifier to distinguish right- and left-hand imaginary

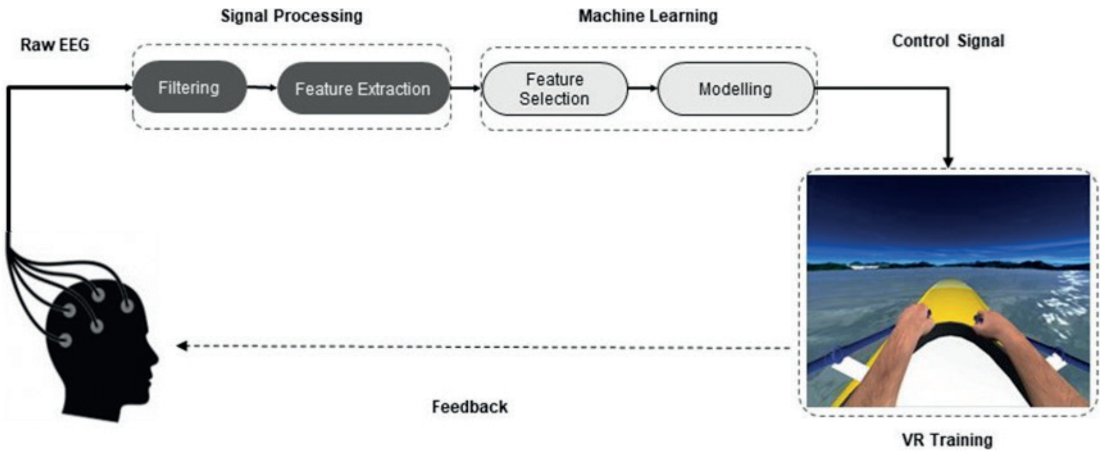

Fig. 2. Motor imagery-based neurofeedback with immersive virtual reality (VR) protocol. Adapted from Vourvopoulos et al. Effects of a Brain-computer interface with virtual reality (VR) neurofeedback: a pilot study in chronic stroke patients. Front Hum Neurosci 2019; 13: 210.

movements. This process was carried out by asking the patient to make mental images of the corresponding hand according to the stimuli presented on the screen (Fig. 2). The training session was set up to acquire data in 24 blocks per class (left- or right-hand images) in random order. Subsequently, the data was spatially and temporally filtered between the Alpha and Beta bands (8–30 Hz) to create the feature vector.

The training was carried out in 12 sessions (3 days a week, for 4 weeks) of 30 min each, divided into 3 series of 7 min (26).

Experimental procedure

The interventions were organized as follows: therapy A consisted in bilateral rTMS protocol exclusively (10 consecutive daily sessions over 2 weeks), whereas therapy B consisted in a combination of the bilateral rTMS protocol and the MI-NFB training. During therapy B the patient received 10 consecutive daily sessions of bilateral rTMS (Monday to Friday, 2 weeks), with the same stimulation parameters as therapy A, and 12 non-consecutive sessions of MI-neurofeedback (3 times a week for 4 weeks). The first 6 MI-NFB sessions were carried out after bilateral stimulation with rTMS (i.e. rTMS as a priming method during the first 2 weeks), and the last 6 sessions, without rTMS as prior priming during the last 2 weeks.

The clinical trial followed an AB/BA crossover design with counterbalanced assignment, in which the first 50% of the sample was assigned to order AB and the second 50% to order BA. The washout period between therapies A and B was always 1 month (Fig. 3).

Participants were assessed 6 times (M1, M2, M3, M4, M5 and M6), 3 assessments per therapy: an initial evaluation prior to the intervention, a second evaluation in the week after the end of the intervention, and a final evaluation 2 weeks later (Fig. 3).

Throughout the study participation period, patients continued with their usual therapies and neither the dosage of content of these therapies changed during their participation period. These therapies were not homogeneous among participants and included neuropsychology, occupational therapy, physiotherapy, and speech therapy, if needed. None of the therapies was initiated or had changed dosing or frequency during the 2 weeks before inclusion.

All interventions took place in a single laboratory where the temperature was controlled to ensure patient comfort. During sessions, patients were accompanied only by the therapist and were permitted to wear comfortable clothing. To minimize auditory discomfort, ear plugs were provided to patients during the rTMS procedure.

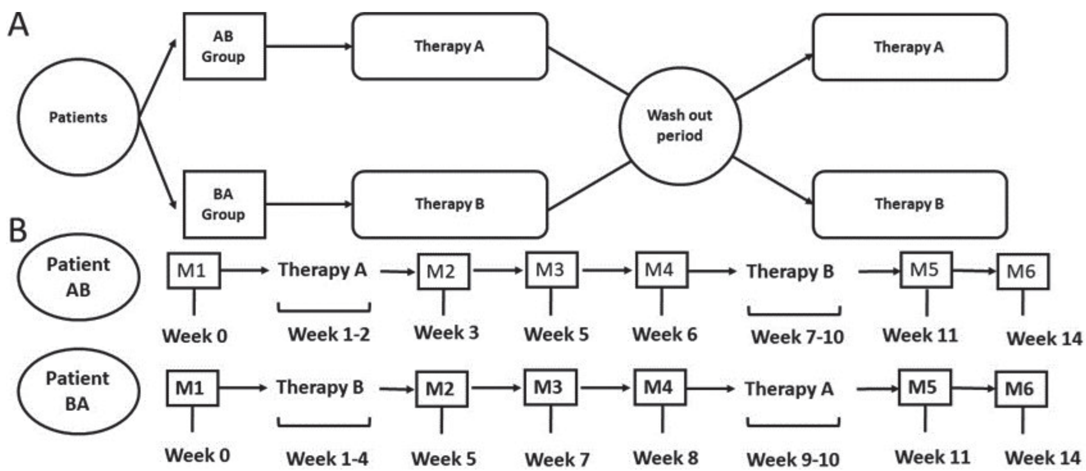

Fig. 3. Study design. (A) Therapy sequence in each group, where therapy A is repetitive transcranial magnetic stimulation alone and therapy B is repetitive transcranial magnetic stimulation plus motor imagery-based neurofeedback training. (B) Evaluation timeline. M1–6: measurements.

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Primary outcome measures

Fugl-Meyer Upper Limb Assessment. The FMA-UL is an observational scale that assesses sensorimotor deficits in post-stroke patients. It includes 4 subscales that make up a total maximum of 66 points. It constitutes the most optimal, sensitive, and appropriate upper limb functionality evaluation scale (27, 28).

Hand Grip Strength. An analogue hand dynamometer (Jamar® Plus+ Hand Dynamometer, 0–90 kg; Performance Health Supply, Nottinghamshire, UK) was used to assess isometric grip strength. The patients sat upright, with both feet on the floor and the forearm resting on a stable surface. The patients performed a maximal isometric grip contraction until they reached maximum force production. The mean value of 3 attempts per hand was recorded (29).

Nottingham Sensory Assessment. The NSA was used to assess UL somatosensory impairment. It is a discrete quantitative tool that measures, analyses, and interprets the individual's reactions to a range of sensory stimuli. This scale evaluates tactile sensation, kinaesthetic sensation and stereognosis (30, 31).

Secondary outcome measures

Nine-Hole Peg Test. The 9-HPT assesses fine manual dexterity. The participants were instructed to pick up 9 pegs from a container as quickly as possible and transfer them, 1 by 1, to a pegboard with 9 holes. The test was performed with both hands and the time spent completing the task was noted (32). The 9-HPT is a reliable, valid, and sensitive tool for measuring change among stroke patients (27, 33).

Computerized Finger Tapping Task. The FTT test measures motor function and speed of signal processing. Participants sat comfortably in front of a computer and pressed the space bar on the keyboard as quickly as possible and repeatedly with the index finger. Five 10-s attempts were made with the unaffected hand. The mean time between 2 consecutive touches in the 5 attempts was noted (34).

Arm Motricity Index. The AMI is a discrete quantitative scale to evaluate muscle strength in 3 actions: pinch, elbow flexion, and shoulder abduction. Each movement is scored, obtaining a total score for the upper limb ranging from 0 (severely affected) to 100 (normal) (35, 36).

Statistical analysis

Statistical analysis was performed in SPSS 26.0 (SPSS Inc., Chicago, IL, USA) and JASP 0.17.2 (JASP Team 2023). For all analyses, a confidence level of 0.95 (alpha = 0.05) was adopted and 95% confidence intervals (95% CI) were obtained. Prior to hypothesis testing, normality was assured with Shapiro–Wilk test and equality of variances between groups with Levene's test.

The presence of carry-over effects was first assessed using repeated measures analysis of variance (ANOVA). Therapy effects were assessed using a linear mixed-effects model (LMM) with therapy, time, and sequence (AB/BA) as fixed-effects and participants as random-effects. Although the sample size was initially calculated for a paired samples *t*-test, it was decided to use a LMM, as it allows accommodation of fixed effects considering the inter-subject variability in the model, and therefore is more flexible.

No modifications were made to the methods employed from the inception of the study to its conclusion.

RESULTS

Participant characteristics

Patient recruitment spanned a period of 18 months, commencing in September 2021 and concluding in February 2023. The recruitment phase ceased upon reaching the calculated sample size. Participant flow is shown in Fig. 1. From 23 volunteers initially screened,

Table I. Demographic and clinical characteristics at baseline

Characteristics	AB group ($n = 10$)	BA group ($n = 10$)	*p*-value
Age, years, mean (SD)	68.50 (9.02)	62.30 (8.32)	0.128
Sex, *n* (%)			0.371
Female	6 (60)	4 (40)	
Male	4 (40)	6 (60)	
Time since stroke, months, mean (SD)	27 (21.01)	27.70 (35.51)	0.958
Stroke type, *n* (%)			0.606
Ischaemic	7 (70)	8 (80)	
Haemorrhagic	3 (30)	2 (20)	
Affected structure, *n* (%)			0.541
Cortical	6 (60)	7 (70)	
Subcortical	4 (40)	3 (30)	
Dominance, *n* (%)			0.305
Right	10 (100)	9 (90)	
Left	0 (0)	1 (10)	
Affected hemisphere, *n* (%)			0.178
Right	3 (30)	6 (60)	
Left	7 (70)	4 (40)	
Montreal Cognitive Assessment, mean (SD)	24.16 (5.08)	23.40 (3.97)	0.115
Fugl Meyer Assessment-Upper Limb, mean (SD)	24.50 (16.21)	24.50 (11.84)	0.513
Hand-grip strength, kg, mean (SD)	4.03 (4.34)	4.87 (4.73)	0.677
Nine Hole Peg Test, s, mean (SD)	167.39 (94.92)	180.75 (103.76)	0.626
Arm Motricity Index, mean (SD)	42.10 (21.60)	42.40 (22.64)	0.852
Finger Tapping Test, s, mean (SD)	586.05 (802.16)	1921.54 (2803.82)	0.206
Nottingham Sensory Assessment-Tactile Sensations, mean (SD)	1.49 (0.57)	1.34 (0.36)	0.947
Nottingham Sensory Assessment-Kinesthetic Sensations, mean (SD)	1.30 (0.53)	1.75 (0.48)	0.051
Nottingham Sensory Assessment-Stereognosis, mean (SD)	4.49 (3.89)	5.16 (4.05)	0.674

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20 participants completed the study and were finally analysed. Participants' characteristics at baseline are summarized in Table I and did not differ between groups ($p>0.05$). Shapiro–Wilk normality test and Levene's test did not show statistical significance in the outcome measures used ($p>0.05$). No adverse effects were reported in patients because of either of the therapies administered.

Primary outcome measures

The different values obtained for each outcome measure and time of measurement are shown in Table II.

Fugl-Meyer Upper Limb Assessment. FMA-UL was the only variable that showed a significant carry-over effect ($p=0.03$). LMM showed significant effects for the therapy factor for FMA-UL ($F\,(1, 64)=27.096$; $p<0.001$) and factor time ($F\,(5, 90)=39.246$; $p<0.001$). No effects were found for the factor sequence ($F\,(1, 18)=1.324$; $p>0.05$). Significant effects were found for sequence by time ($F\,(5, 90)=9.164$; $p<0.001$). Post-hoc analyses showed significant differences at measurement 2 (E=4.433; t=5.385; $p<0.001$), 3 (E=–2.217; t=–2.692; $p=0.008$), 4 (E=–2.217; t=–2.692; $p=0.008$) and 5 (E=–2.267; t=–2.753; $p=0.007$) in favour of therapy B (Fig. 4A). No other measurements showed significant differences ($p>0.05$).

Hand Grip Strength. The LMM did not show significant outcomes for the therapy factor ($F\,(1, 18)=0.311$; $p>0.05$) and the sequence factor ($F\,(1, 18)=1.053$; $p>0.05$) concerning Hand Grip Strength. However, significant effects were observed for the time factor ($F\,(5, 90)=3.127$; $p<0.001$). A significant interaction between sequence and time was not detected ($F\,(5, 90)=1.823$; $p>0.05$). Subsequent post-hoc analyses showed significant differences at measurement 2 (E=0.862; t=2.030; $p=0.045$) and 3 (E=–1.036; t=–2.440; $p=0.017$) favouring therapy B (Fig. 4B). No significant variations were observed in the remaining measurements ($p>0.05$).

Nottingham Sensory Assessment (NSA)

- *Tactile Sensation (NSA-TS).* Significant effects were observed for the therapy factor ($F\,(1, 18)=7.065$; $p=0.016$), as well as for the time factor ($F\,(5, 90)=7.130$; $p<0.001$). No significant effects were found for the sequence factor ($F\,(1, 18)=0.557$; $p>0.05$). A significant interaction effect was observed between sequence and time ($F\,(5, 90)=6.759$; $p<0.001$). There were significant differences at all measurements ($p<0.05$) in favour of therapy B (Fig. 5A).
- *Kinesthetic Sensation (NSA-KS).* Significant effects were observed for the therapy factor ($F\,(1, 18)=9.036$; $p=0.004$), as well as for the time factor ($F\,(5, 90)=11.146$; $p<0.001$) and sequence factor ($F\,(1, 18)=7.647$; $p=0.013$). A significant interaction effect was observed between sequence and time ($F\,(5, 90)=15.557$; $p<0.001$). There were significant differences at all measurements ($p<0.05$) in favour of therapy B except at time 2 (E=0.066; t=1.156; $p>0.05$) (Fig. 5B).

Table II. Values of outcome measures by time and sequence of treatment

	Measurement					
Variable	1	2	3	4	5	6
FMA-UL, mean (SD)						
AB	24.50 (16.21)	29.20 (18.13)	29.20 (17.35)	28.80 (16.59)	36.50 (16.41)	36.70 (16.64)
BA	24.50 (11.84)	42.50 (18.51)	42.50 (19.50)	42.20 (19.16)	43.50 (18.78)	42.90 (20.53)
Hand-grip strength, kg, mean (SD)						
AB	4.03 (4.34)	4.47 (4.31)	4.16 (4.37)	4.03 (4.10)	5.18 (4.74)	5.04 (4.37)
BA	4.87 (4.73)	9.10 (10.47)	7.18 (6.13)	6.54 (6.11)	7.13 (6.21)	7.44 (7.39)
NSA-TS, mean (SD)						
AB	1.49 (0.57)	1.45 (0.60)	1.36 (0.57)	1.30 (0.55)	1.77 (0.48)	1.76 (0.42)
BA	1.34 (0.36)	1.76 (0.38)	1.74 (0.39)	1.77 (0.69)	1.66 (0.59)	1.70 (0.50)
NSA-KS, mean (SD)						
AB	1.30 (0.53)	1.35 (0.47)	1.35 (0.47)	1.22 (0.47)	2.12 (0.77)	1.98 (0.52)
BA	1.75 (0.48)	2.30 (0.42)	2.37 (0.56)	2.27 (0.55)	2.17 (0.60)	1.95 (0.43)
NSA-S, mean (SD)						
AB	4.49 (3.89)	4.50 (3.89)	4.40 (3.97)	4.40 (3.97)	4.63 (3.77)	4.53 (3.86)
BA	5.16 (4.05)	5.46 (3.72)	4.76 (3.64)	4.63 (3.76)	4.70 (3.71)	4.76 (3.64)
9-HPT, s, mean (SD)						
AB	167.39 (94.92)	149.23 (91.20)	137.52 (93.14)	153.02 (106.54)	138.86 (99.65)	124.64 (96.33)
BA	180.75 (103.76)	151.04 (81.72)	167.72 (103.40)	171.71 (139.92)	146.27 (121.17)	107.75 (91.84)
AMI, mean (SD)						
AB	42.10 (21.60)	50.80 (22.30)	48.50 (25.35)	40.40 (24.51)	65.70 (26.00)	64.10 (29.18)
BA	42.40 (22.64)	70.70 (23.99)	67.70 (29.22)	66.20 (28.06)	70.80 (23.60)	70.00 (24.50)
FTT, ms, mean (SD)						
AB	586.05 (802.16)	520.14 (640.96)	633.13 (649.71)	708.34 (840.04)	2,827.66 (6,893.94)	670.66 (598.86)
BA	1,921.54 (2,803.82)	2,734.07 (3,700.31)	1,738.35 (3,156.39)	1,536.39 (3,076.39)	514.58 (464.81)	469.29 (674.84)

Arm MI: Arm Motricity Index; FMA-UL: Fugl-Meyer Assessment-Upper Limb; FTT: Finger Tapping Test; 9-HPT: Nine-Hole Peg Test; NSA-TS: Nottingham Sensory Assessment-Tactile Sensations; NSA-KS: Nottingham Sensory Assessment-Kinesthetic Sensations; NSA-S: Nottingham Sensory Assessment-Stereognosis; SD: standard deviation.

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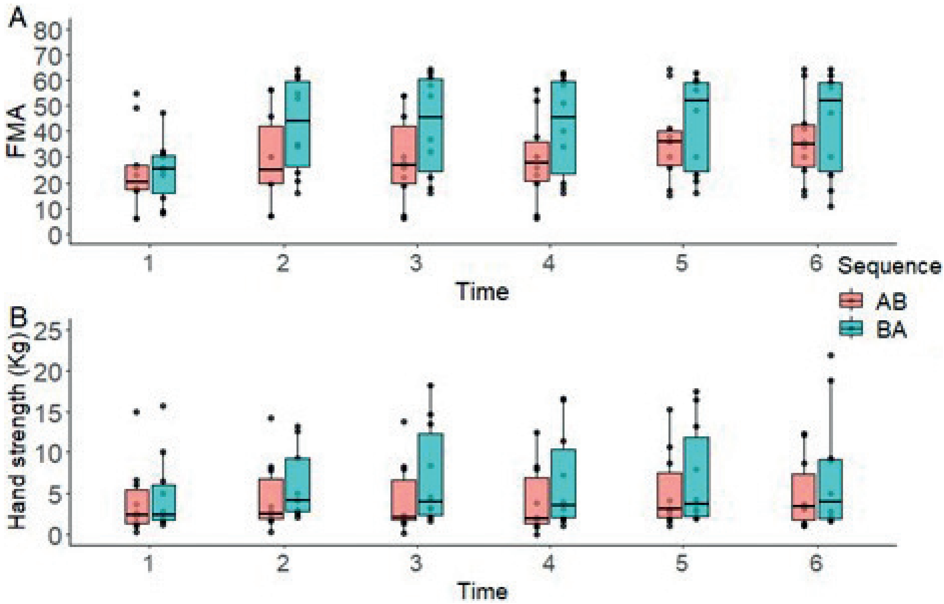

Fig. 4. Changes in (A) Upper Limb Fugl-Meyer Assessment (FMA-UL) and (B) Hand-Grip Strength (Kg) over time, according to the treatment sequence.

- *Stereognosis (NSA-S).* No significant effects were observed for the therapy factor (F (1, 18)=0.636; $p>0.05$), the time factor (F (5, 90)=1.009; $p>0.05$), sequence factor (F (1, 18)=0.063; $p>0.05$), as well as for sequence and time interaction (F (5, 90)=0.905; $p>0.05$). There were no significant differences at any measurements ($p>0.05$) (Fig. 5C).

Secondary outcome measures

The therapy factor showed significant effects on the AMI (F (1, 93)=13.021; $p<0.001$). The sequence factor had no significant effect on the changes produced by therapies in any outcome measure ($p>0.05$). Both AMI and 9-HPT exhibited significant effects for the time factor (F (5, 90)=17.556; $p<0.001$) and (F (5, 65)=3.097; $p=0.014$), respectively). Regarding the interaction between the sequence and time factors, significant effects were only found in AMI (F (5, 90)=4.949; $p<0.001$). Only measurements 1 (E=2.300; t=2.953; $p=0.004$) and 2 (E=–6550; t=–3120; $p=0.002$) showed significant changes in favour of therapy B. No significant effects were found for any of the factors on the FTT.

DISCUSSION

This study investigated whether the combined application of bilateral rTMS and MI-based NFB training improves UL motor function to a greater extent than rTMS alone in subacute and chronic post-stroke patients. A significant improvement was observed in UL motor function (FMA-UL), favouring the combined protocol (rTMS + MI-based NFB). However, contrary to expectations, hand-grip strength did not show significant between-therapy differences. For the somatosensory deficits (NSA), improvements were notable particularly in tactile and kinaesthetic sensation, again favouring the combined therapy, although without differences in stereognosis. In addition, the combined approach also had a positive impact on secondary outcome measures, such as AMI. Altogether, these results support the hypothesis of the study.

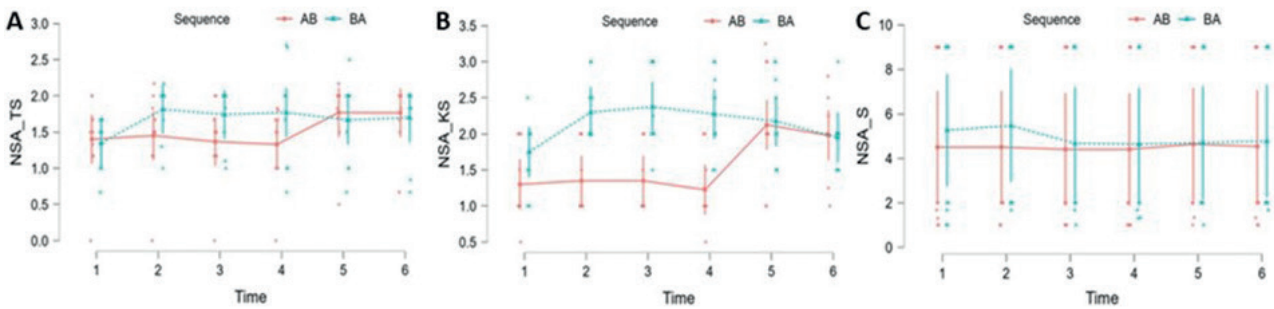

Fig. 5. Changes in Nottingham Sensory Assessment (NSA): (A) Tactile Sensation (TS), (B) Kinesthetic Sensation (KS) and (C) Stereognosis (S) over time, according to the treatment sequence.

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The FMA-UL was the only variable that presented a significant carry-over effect, suggesting that the effects of rTMS were maintained beyond the intervention period. As all participants continued their conventional rehabilitation therapies during the washout month, it cannot be ruled out that this could have influenced the permanence of the effects.

Beneficial effects of combining rTMS and BCI training have been proved in previous studies; our findings confirm this idea in general, but also provides a broader understanding of clinical efficacy of this specific combination. One such study, by Johnsson et al. (37), compared a 2-week combined protocol of rTMS and BCI training against sham-rTMS in a cross-over design ($n=3$). Their methods diverged from ours in terms of rTMS parameters (wherein they utilized low frequency on the unaffected side, at 1 Hz and 90% RMT for 10 min over 9 sessions, in contrast to our 10-session bilateral protocol) and a variant form of BCI training (centred on opening and closing of the hand throughout 18 sessions, with the initial 9 primed by rTMS and the remaining 9 without). Their outcomes showed significant enhancements in gross manual dexterity (Box and Blocks test) in the sham rTMS+BCI condition and in both conditions for the FTT test on the affected side proving a beneficial effect of BCI, but with a clear enhancement by rTMS. In a similar way to Johnsson the current study also proved the superiority of the combination of both therapies based on improvement of functional scales (FMA-UL), but the current study protocol additionally showed effects on sensory variables.

Conversely, Chen et al. (38) used iTBS on the affected hemisphere as a primer to VR cycling training for UL compared with sham iTBS as the priming for the same training. This study confirmed the beneficial effects of VR, but a clear enhancement by the iTBS priming was also evident in Modified Ashworth Scale Upper-Extremity, Motor Activity Log and Stroke Impact Scale. Unlike the current study, although Chen et al. (38) showed improvement for FMA-UL, they did not demonstrate significant between-group differences.

Despite the methodological differences, these 2 studies suggest that pre-training rTMS stimulation can modify or enhance the effects of NFB or VR. In a complementary manner, the current study demonstrates that MI-based NFB enhances the effect of bilateral rTMS. The optimal type of rTMS stimulation and the therapy to be combined to, however, remains a topic of debate. Bilateral rTMS application relies on the combined neurophysiological effects derived from both the decreased excitability on the unaffected side (37) and the increased excitability on the affected hemisphere (38).

In the current study, the absence of substantial differences between the therapies and sequence in hand-grip strength implies that both therapeutic strategies are comparably effective in enhancing this metric. It is worth noting that the NFB is based in imagined movement, which is congruent with the observation that, in the NSA, the combined approach showed notable effects on the touch and movement sensation tests (i.e. positive changes in the distal part of the limb), improving both skills. The effectiveness of this strategy in enhancing sensory components, may be attributed to the vibrating touch feedback and the embodiment that NeuRow provides (39, 40).

In terms of secondary variables, a significantly different effect between therapies was observed only in the AMI. This supports, along with the FMA-UL results, the clinical efficacy of the combined approaches in enhancing the functionality of the impaired limb. The 9-HPT showed notable time effects, indicating that patients experienced significant improvements in fine manual dexterity after receiving both therapies, with no discernible differences between them. One possible explanation to this lack of adjuvant effect of MI based- NFB could be a ceiling effect already achieved by rTMS stimulation. We hypothesize that functional brain changes for improving reaching movements involving the shoulder/elbow and distal motor control could rely on different stages of recovery or diverse neural plasticity patterns driving functional recovery (41), which may explain why enhancement of distal improvements in motor components were not observed with the combined treatment. Lastly, neither the brain changes induced by any of the interventions appeared to alter participants' brain processing speed, measured with a FTT paradigm on the unaffected side. This could imply a ceiling effect for FTT on the unaffected hemisphere, but also implies that bilateral rTMS did not disrupt the function of the non-affected hemisphere in terms of FTT and possibly cognitive processing speed.

Limitations

This study has some limitations. First, it is important to acknowledge that the study was not conducted using a randomized double-blind approach. While the results are certainly significant and important, caution is required when extending these results to broader contexts. Secondly, there was a difference in the duration of the rTMS and NFB neuromodulation protocols (10 sessions in 2 weeks and 12 sessions in 4 weeks, respectively). This discrepancy, which is due to the design of the study based on previous neuromodulation studies (21, 26), could have affected the results. The assessments were carried out immediately after the whole intervention finished. This might have missed midterm changes of the rTMS treatment for the group

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receiving therapy A, or immediate effects from rTMS in the therapy B group.

The absence of corrections for multiple comparisons poses a potential limitation, which might affect the risk of obtaining false-positive results. In addition, given that each participant underwent the evaluation battery 6 times, the study did not account for potential learning effects, which might influence the outcomes across repeated assessments.

Finally, the participants continued with their usual neurorehabilitation routine during the treatment, which was not controlled in this research. However, it is important to note that these routines remained consistent for each participant throughout the study period, minimizing their impact on the study of treatment effects due to the crossover design.

This study demonstrates several key findings. MI-based NFB can amplify the impact of bilateral rTMS on the functional evaluation and somatosensory perception of the upper limb, boosting the effects of conventional therapy. Nonetheless, this interaction may also be indicative of a potential priming effect of rTMS on NFB efficacy. This finding supports the previous reports of adjuvant effects of rTMS to other therapeutic interventions for post-stroke motor recovery.

Although the study was not explicitly structured to assess the persistence of the effects of the interventions, the observed carryover effect suggests a lasting effect of at least 1 month after bilateral rTMS intervention in both groups.

There is a differential impact of MI-based NFB on UL functionality potentiation vs strength, which may indicate a specific effect of motor imagery add on in contrast with other strategies used in different studies with also different results. This reinforces the need for personalized therapy plans.

The study's objective was not to investigate the safety of bilateral rTMS stimulation with injured hemisphere stimulation and unaffected hemisphere inhibition. However, the current findings confirm that it is a safe procedure. There was no decline in function due to inhibition of the unaffected hemisphere, nor were there adverse effects from stimulating the affected side.

Conclusion

These findings suggest that rTMS is a promising treatment for the rehabilitation of stroke patients in both subacute and chronic phases. Combining rTMS with other non-invasive neuromodulation strategies, such as neurofeedback (NFB) and virtual reality (VR), should be tailored to target specific rehabilitation goals, whether to improve dexterity, sensitivity, or strength. The use of these combined therapies in clinical settings could significantly impact a large subset of patients, particularly where therapeutic options are limited, as in the subacute and chronic phases. Further validation of the trends observed in this exploratory study is necessary, using a randomized clinical trial.

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The study was conducted in accordance with the Declaration of Helsinki; the protocol was approved by the Ethics Committee of Hospital de Fuenlabrada on 26 June 2019 (project identification code 19-11).

The study has been registered in clinicaltrials.gov (number NCT04815486).

The authors have no conflicts of interest to declare.

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To whom It may concern,

I am pleased to confirm that Mr. Francisco José Sánchez Cuesta, with identity number 47344012F has completed a predoctoral research internship at the Laboratory of Evolutionary Systems and Biomedical Engineering (LaSEEB), of the Institute of Systems and Robotics - ISR-Lisboa, in the period between 04/01/2023 and 07/01/2023.

Mr. Francisco José Sánchez Cuesta is currently a PhD student in the PhD Program in Medicine, Biotechnology and Biomedical Sciences of the Faculty of Experimental Sciences of the Francisco de Vitoria University, Madrid.

During his stay, he carried out research on the interpretation of EEG signals recorded during neuromodulation interventions for the rehabilitation of the affected upper limb after stroke, under my supervision in order to achieve the objectives established in his doctoral work plan.

Sincerely,

Athanasios Vourvopoulos