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Faculty of Heath Sciences

Doctoral Dissertation

THE IMPACT OF IN-PHASE BILATERAL UPPER LIMB EXERCISES IN PEOPLE WITH MULTIPLE SCLEROSIS

Dimitris Sokratous

Limassol, April 2025

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Approval Form

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To my dear Stylianos: I truly hope you grow to be much smarter than me and achieve all your dreams. I hope that this shared journey and the lessons learned along the way will serve as a guiding light and inspiration for you in everything you do.

ABSTRACT

Multiple Sclerosis is an autoimmune disease of the central nervous system, marked by relapses, progressive neurological deterioration, or both. The predominant clinical symptoms include motor dysfunction and cognitive deficits. Relapsing-Remitting Multiple Sclerosis primarily involves motor impairments due to corticospinal tract dysfunction, while Progressive Multiple Sclerosis is associated with significant cognitive and motor decline, often beginning with cognitive processing deficits often appearing first. This thesis examined the effects of in-phase bilateral upper limb exercises on neurophysiological, cognitive, and clinical outcomes in people with Multiple Sclerosis. A 12-week program (30 – 60 minutes/session, three sessions/week) incorporated sports-based and functional motor training. Two clinical trials were conducted across different phenotypes of Multiple Sclerosis. The first clinical trial study used a single-case design with one Relapsing-Remitting Multiple Sclerosis participant, followed by a multiple baseline study with five participants. Visual and statistical analyses assessed intervention effects on corticospinal plasticity, motor and cognitive measures. The second clinical trial study included 20 participants with Progressive Multiple Sclerosis allocated into an experimental group performing in-phase bilateral exercises and into an active control group following conventional exercises. Motor and cognitive improvements were observed using repeated measures ANOVA with Post Hoc Bonferroni corrections. Results showed bilateral reductions in resting motor threshold, measured by Transcranial Magnetic Stimulation, and improvements in motor and cognitive functions in Relapsing-Remitting Multiple Sclerosis. In Progressive Multiple Sclerosis, the experimental group showed significant improvement in motor and cognitive functions compared to the active control group. These findings suggest that in-phase bilateral exercises enhance cortical excitability in Relapsing-Remitting Multiple Sclerosis and improve motor and cognitive functions across people with Multiple Sclerosis in general. This thesis supports in-phase bilateral exercises as an effective neurorehabilitation strategy. Further research should refine methodologies to maximize intervention efficacy.

Keywords: Multiple Sclerosis, corticospinal plasticity, Transcranial Magnetic Stimulation, cognitive processing in-phase bilateral, exercise

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List of Abbreviations

MS	Multiple Sclerosis
CNS	Central Nervous System
pwMS	People with Multiple Sclerosis
RRMS	Relapsing-Remitting Multiple Sclerosis
PMS	Progressive Multiple Sclerosis
pwRRMS	People with Relapsing-Remitting Multiple Sclerosis
pwPMS	People with Progressive Multiple Sclerosis
rMT	resting Motor Threshold
SMA	Supplementary Motor Area
M1	Primary Motor Cortex
QoL	Quality of Life
MRI	Magnetic Resonance Imaging
ADL	Activities of Daily Living
EDSS	Expanded Disability Status Scale
MEPs	Motor Evoked Potentials
EMG	Electromyography
CMCT	Central Motor Conduction Time
%MSO	Percentage of Maximum Stimulator Output
SD	Standard Deviation
PEM	Percentage of data Exceeding the Median
NAP	Nonoverlap of All Pairs
HR	Heart Rate
RPE	Rate of Perceived Exertion
PNF	Proprioceptive Neuromuscular Facilitation Technique

CHAPTER 1:

General Introduction

1.1 Burden of Neurological Disorders

Neurological disorders are a major contributor to premature mortality and can result in either transient or permanent disability. Chronic neurological diseases, in particular, are among the most significant public health challenges worldwide. Due to their progressive nature and long-term impact, individuals with these conditions require lifelong medical and rehabilitative support to maintain their quality of life (QoL).

The socioeconomic burden of these disorders extends beyond direct medical costs, as they also lead to reduced productivity, increased caregiver responsibilities and significant emotional distress for both patients and their families. As populations age and life expectancy increases, the prevalence of neurological conditions is expected to rise, placing additional strain on healthcare systems and highlighting the urgent need for effective interventions.

Neurorehabilitation and exercise play a critical role in managing these conditions by promoting functional recovery, enhancing independence and optimizing overall well-being. In this context, qualitative research is essential for gaining deeper insights into patients' lived experiences, refining personalized treatment approaches and improving rehabilitation and exercise strategies. By understanding the challenges faced by individuals with chronic neurological disorders, healthcare professionals can develop more tailored, patient-centered interventions that address not only physical impairments but also cognitive, psychological, and social factors influencing recovery and adaptation.

1.2 Multiple Sclerosis: Definition, Pathophysiology, and Clinical Manifestation

Among the various chronic neurological diseases, Multiple Sclerosis (MS) is one of the most common. MS is an autoimmune, inflammatory disease of the central nervous system (CNS) characterized by demyelination and neurodegeneration. It manifests in different forms, including episodic periods of worsening (acute relapses), gradual progressive deterioration of neurological function, or a combination of both (Lublin & Reingold, 1996). MS occurs due to autoimmune attacks on myelinated axons, leading to demyelination, which in turn results in axonal degeneration or neurodegeneration (Trapp & Nave, 2008). The pathological hallmark of MS is the formation of inflammatory focal

lesions (plaques) in the white matter of the brain and spinal cord, as well as in the grey matter, including the cortex, basal ganglia, brainstem, and spinal cord (Ghasemi et al., 2017). MS is defined by the presence of these sclerotic plaques (Brück, 2005), which lead to motor and cognitive deficits (Ludwin, 2006).

1.3 Detailed Pathophysiology of Multiple Sclerosis

1.3.1 Mechanisms of demyelination

Demyelination is the primary pathological feature of an MS plaque, caused by the destruction of both myelin and oligodendrocytes due to autoimmune attacks. During the demyelination process, the blood–brain barrier is compromised, leading to focal infiltrations of T cells and macrophages through astrocytes. This process also triggers gliosis and the death of oligodendrocytes (Figure 1.1) (Caramia et al., 2004; Louapre & Lubetzki, 2015).

During an immune attack, both cellular and humoral immunity are activated, involving T and B cells, respectively. T cells target myelin and oligodendrocytes, leading to phagocytosis by macrophages. Meanwhile, B cells secrete anti-myelin antibodies, resulting in the opsonization of myelin and oligodendrocytes, which are subsequently degraded by macrophages (Bando, 2020; Brück, 2005).

The inflammatory lesions along the myelin sheath impair nerve conduction, causing a delay in the transmission of nerve impulses. This impairment is a hallmark of the relapsing stage in people with MS (pwMS) (Correale & Marrodan, 2019). However, conduction failure can also arise from molecular damage to the demyelinated fibre itself, leading to progressive degeneration. Axonal degeneration contributes to brain atrophy and loss of brain tissue, forming the structural basis for permanent impairments observed in the progressive stage of the disease (Mahad et al., 2015).

Figure 1.1. Pathogenesis of a Multiple Sclerosis lesion.

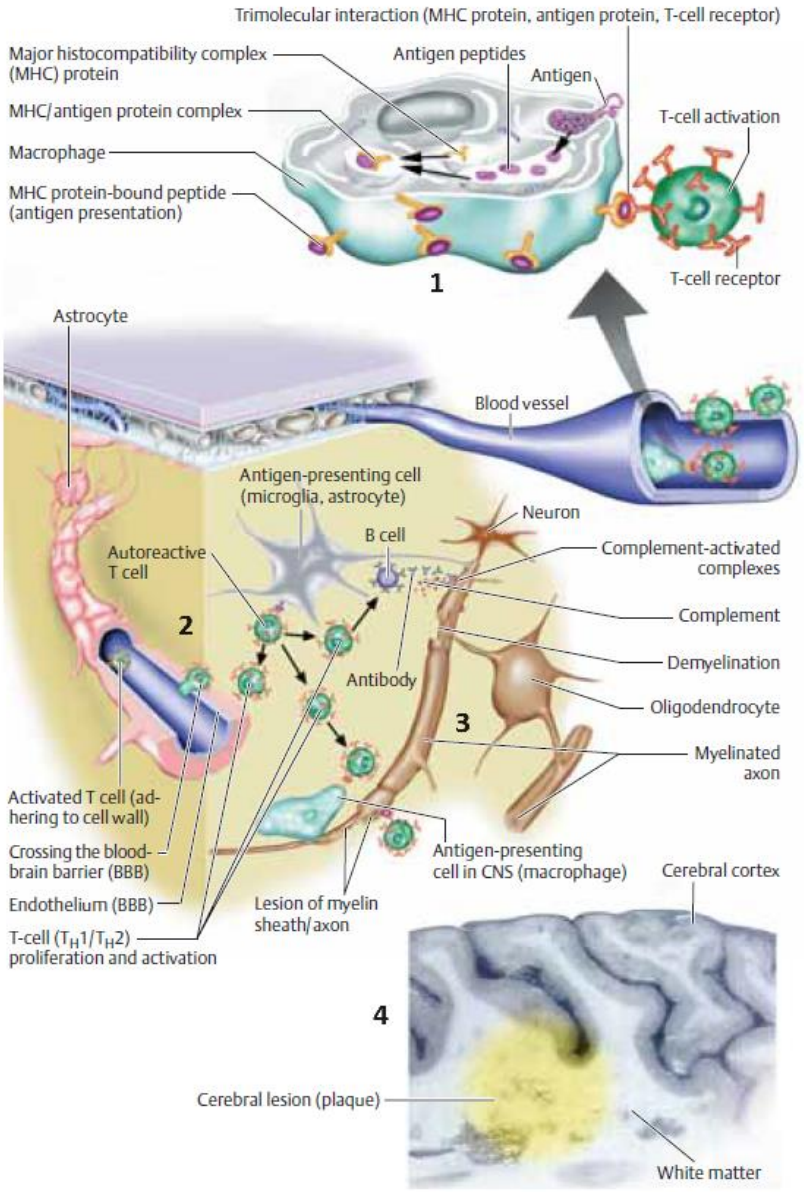


Figure 1.1. MHC; Major Histocompatibility Complex, CNS; Central Nervous System, BBB; Blood-Brain Barrier. 1. Macrophage - T-cell interaction. 2. Crossing of the Blood-Brain Barrier. 3. T and B cells activation targeting the oligodendrocytes and the myelinated axons. 4. Multiple Sclerosis plaque.

MS plaques are defined as demyelinated areas that evolve differently during the early and chronic stages of the disease. Several processes contribute to plaque formation, including inflammation, myelin breakdown, astrogliosis, oligodendrocyte damage, neurodegeneration, and potential remyelination. Plaques are typically categorized as acute active, chronic active, chronic inactive, or remyelinated shadow plaques.

The primary distinction between active and inactive plaques lies in the local activation of macrophages. Acute active lesions are characterized by active demyelination and inflammatory infiltration. In contrast, chronic active lesions involve demyelinated areas with little or no inflammatory infiltration, while chronic inactive lesions are completely demyelinated and lack macrophages or microglia (Lassmann, 2018; Ludwin, 2006). Shadow plaques result from remyelination and are characterized by thinly myelinated axons. Remyelination is more common in Relapsing-Remitting Multiple Sclerosis (RRMS) but can also occur in small regions at the edges of chronic inactive plaques during the progressive stage. However, because remyelination depends on the availability of oligodendrocytes, it is often transient, and shadow plaques may undergo renewed demyelination (Lassmann, 2018; Patrikios et al., 2006).

Traditionally, MS was considered a white matter disease due to the multifocal areas of inflammatory demyelination in white matter. However, inflammatory plaques, transected axons and astrogliosis are also present in the grey matter of the CNS (Calabrese et al., 2010; Ghasemi et al., 2017). Grey matter demyelination is associated with the loss of axons, neurons and glial cells, along with a significant reduction in synapses. Remyelination is more extensive in grey matter compared to white matter, though inflammation, macrophage recruitment and microglial activation are less pronounced. These grey matter changes often lead to cortical atrophy, contributing to motor impairments and cognitive decline (Correale & Marrodan, 2019).

1.3.2 Mechanisms of neurodegeneration

Neurodegeneration has an essential role in the progressive stage of MS and is a strong predictor of the individual clinical disability (Levin et al., 2014). Although MS is more prevalent in women than men (3:1) (Portaccio et al., 2024), the female-to-male ratio is lower in Progressive MS (PMS), with grey matter atrophy and cognitive dysfunctions more commonly observed in male patients (Schoonheim et al., 2012).

While axonal degeneration and neuronal loss are hallmark features of the progressive stage, neurodegeneration also occurs during the relapsing stage, affecting many grey matter regions (Louapre & Lubetzki, 2015). Neurodegeneration was believed to result from demyelination triggered by inflammation. However, Levin et al. (2014), suggest that

neurodegeneration can occur prior to demyelination, indicating that these processes may be independent pathological mechanisms (Levin et al., 2014).

As MS is a complex and heterogeneous disease, several mechanisms may contribute to neurodegeneration, with the most prominent theories involving hypoxia, oxidative stress, autoantibodies and metabolic disturbances. **Hypoxia** occurs in chronically demyelinated neurons, which is associated with the limited production of adenosine triphosphate and with ion channel and mitochondrial dysfunction. The resulting energy deficit leads to neuronal death. Hypoxia is often observed in tissues at sites of chronic inflammation, which may contribute to MS plaque formation. **Oxidative stress** is driven by the production of reactive oxygen species, secreted by macrophages. **Reactive oxygen species** are free radicals or molecules with unpaired electrons (e.g., hydroxyl radical, superoxide radical, nitric oxide), which act as electron acceptors and induce oxidation. This process damages DNA, proteins and lipids, ultimately leading to cell death through necrosis or apoptosis (Levin et al., 2014).

Neurodegeneration can also be influenced by both myelin-specific and non-myelin autoantibodies. Myelin autoantibodies primarily target proteins such as myelin oligodendrocyte glycoprotein, myelin basic protein, myelin-associated glycoprotein and proteolipid protein, playing a significant role in RRMS. In contrast, non-myelin autoantibodies are more prominent in PMS. These antibodies target neuronal surface molecules, cytoskeletal proteins (e.g., neurofilaments), intracellular enzymes and nuclear antigens (e.g., nuclear ribonucleoproteins).

Both types of autoantibodies contribute to neurodegeneration by activating apoptotic inflammatory cytokines and immune response pathways. Furthermore, metabolic disturbances are thought to exacerbate neurodegeneration by promoting hypoxia and reactive oxygen species activity. Elevated levels of neurotoxic factors such as homocysteine, vitamin B12 deficiency and dyslipidemia can lead to the breakdown of the myelin sheath and subsequent neuronal degeneration (Correale & Marrodan, 2019; Levin et al., 2014).

1.3.3 Etiology of Multiple Sclerosis

Throughout the literature, MS is associated with a complex interaction of genetic, immunologic and environmental factors (Dobson & Giovannoni, 2019). However, a

recent study stated that Epstein–Barr Virus infection suggested to be the leading cause of MS. Epstein–Barr Virus it is a common type of herpes virus, which provokes MS by priming an autoimmune process (Bjornevik et al., 2022). The study of Bjornevick et al. (2022), included 10 million active-duty United States soldiers, of whom 955 were diagnosed with MS and their main feature was Epstein–Barr Virus infection (Figure 1.2). The specific study, reported that serum neurofilament light chain, which is a biomarker of axonal degeneration, increased only after Epstein–Barr Virus seroconversion (Figure 1.3) and nearly 20% to 25% of this sample had antibodies in their blood that bind tightly to both a protein from the Epstein–Barr Virus, named EBNA1 and a protein found in the spinal cord and brain, named the glial cell adhesion molecule (Bjornevik et al., 2022).

Figure 1.2. Epstein–Barr virus infection is associated with markedly higher Multiple Sclerosis risk.

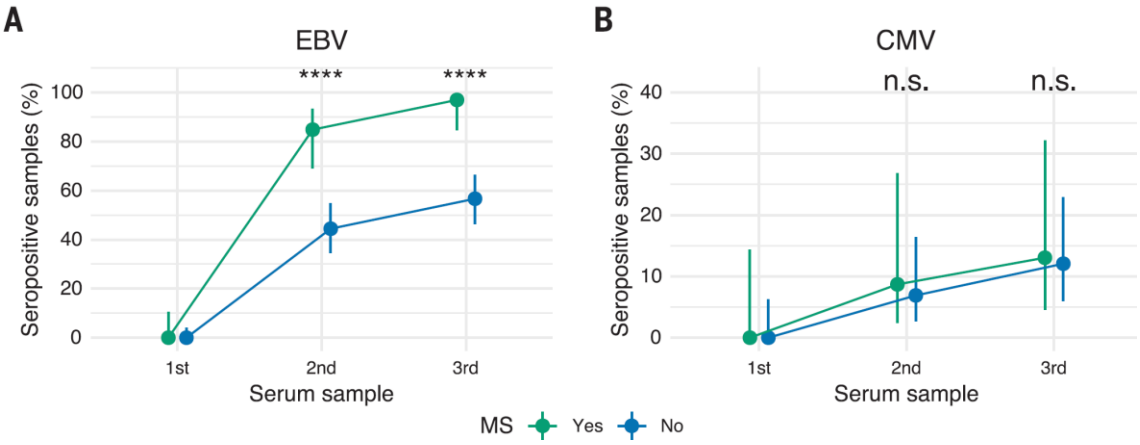


Figure 1.2. EBV; Epstein–Barr virus, CMV; cytomegalovirus. (A) The proportion of persons who were EBV-positive during the first, second, and third sample, was significantly higher in individuals who later developed MS, than those (B) who were CMV-positive (Bjornevik et al., 2022).

Figure 1.3. Epstein–Barr virus infection is associated with elevation of serum neurofilament and with Multiple Sclerosis onset.

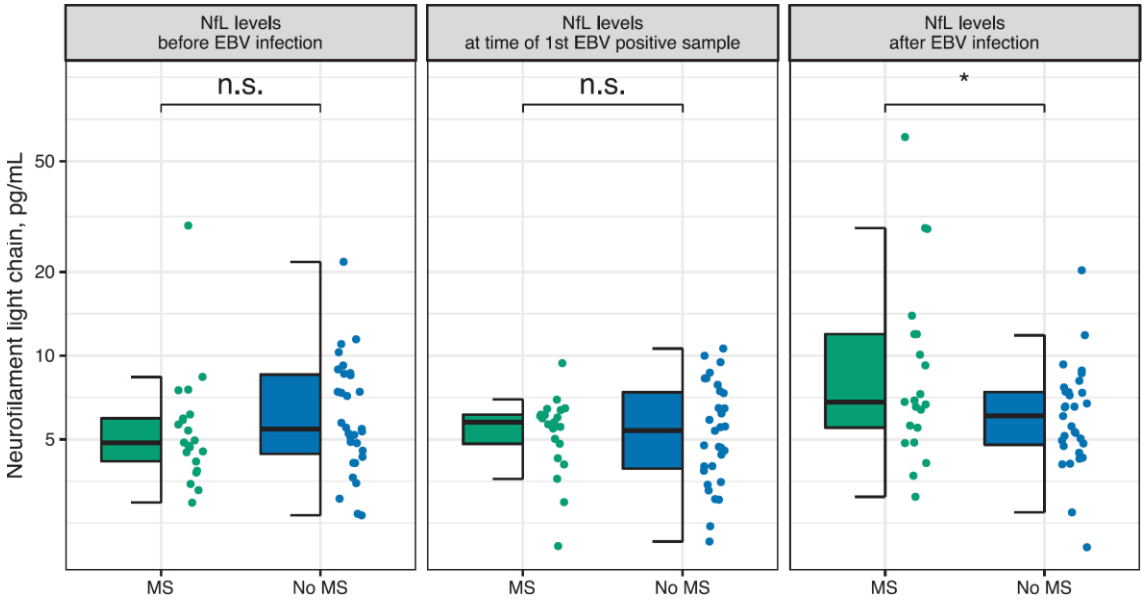


Figure 1.3: NfL; neurofilament, EBV; Epstein–Barr virus, MS; Multiple Sclerosis. A box plots of serum neurofilament levels before, around, and after the time of EBV infection (Bjornevik et al., 2022).

Although, Epstein–Barr Virus infection appears to be a predominant factor in the etiology of MS, several environmental factors also contribute to increased disease susceptibility, including vitamin D deficiency, obesity and smoking (Dobson & Giovannoni, 2019). MS prevalence is notably higher in regions farther from the equator, which is associated with lower ultraviolet B exposure and consequently, reduced vitamin D production. In contrast, individuals living closer to the equator tend to have higher vitamin D levels due to greater ultraviolet B exposure, which is thought to provide some protection against immune-mediated diseases, including MS (Breuer et al., 2019).

Additionally, previous studies have shown that smoking and obesity significantly increase the risk of developing MS. Smoking is associated with faster disease progression, whereas quitting smoking is linked to slower progression and reduced disability (Arneth, 2020; Hedström et al., 2013). Childhood and adolescent obesity are also risk factors for MS and are associated with greater clinical disability (Gianfrancesco & Barcellos, 2016; Stampanoni Bassi, Iezzi, et al., 2020).

Although MS is not considered a hereditary disease, some individuals have a genetic predisposition to it (Olsson et al., 2016). Monozygotic twins have a higher concordance rate (20% – 30%) compared to dizygotic twins (2% – 5%), indicating a significant genetic component. Furthermore, gene-environment interactions appear to influence MS susceptibility. For example, regions with a higher prevalence of MS show higher concordance rates in twins and siblings of an affected individual are 10 – 15 times more likely to develop MS compared to the general population (Canto & Oksenberg, 2018), particularly in the HLA-DRB1 and IL-7R genes. The HLA-DRB1 gene plays a critical role in the immune system by helping to distinguish the body's proteins from those of pathogens. The IL-7R gene encodes receptor proteins that are essential for immune system function. Variations in these genes are linked to damage of the myelin sheath in individuals with MS (Canto & Oksenberg, 2018; Ferrè et al., 2020).

1.4 Epidemiology and Socioeconomic Burden of Multiple Sclerosis

Several epidemiological studies reported that the estimated number of pwMS worldwide is around 2.8 million (Haki et al., 2024; Portaccio et al., 2024; Walton et al., 2020). According to Walton et al. (2020), data collected from 115 countries (representing 87% of the global population) revealed a 30% increase in the estimated number of pwMS in 2020 compared to 2013. In 2020, the global prevalence of MS was reported at 35.9 per 100,000 people. Recent studies also highlight that regions such as the United States, Middle East and North Africa, Russia, Canada, Australia and several European countries have seen a significant rise in MS prevalence compared to previous years (Figure 1.4) (Walton et al., 2020).

Globally, MS has been increasing more rapidly among women, with the female-to-male ratio now at 3:1 (Portaccio et al., 2024). A study by Weber and Clyne (2021), suggested that the higher prevalence in women may be linked to the presence of the Sphingosine-1-Phosphate Receptor 2 protein (Weber & Clyne, 2021). This protein regulates the permeability of the blood-brain barrier and is found to be produced in significantly higher quantities in women, especially in those with MS, compared to healthy individuals.

Figure 1.4. Geographic map of variation in Multiple Sclerosis prevalence.

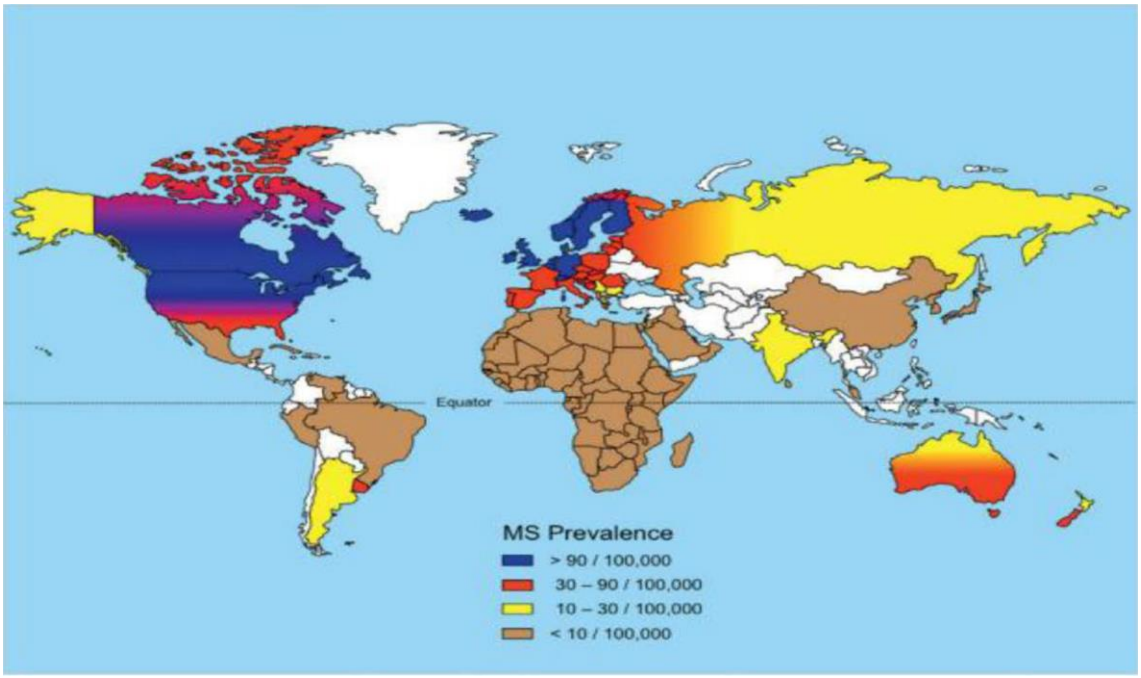


Figure 1.4: MS; Multiple Sclerosis. The prevalence of MS per 100,000 population by world regions country (Haki et al., 2024).

Both women and men with MS have a shorter life expectancy, typically 6 – 10 years less than the general population. The mortality risk for pwMS remains two to three times higher than in the general population (Kingwell et al., 2020). Specifically, people with PMS (pwPMS) have a higher mortality rate compared to those with RRMS and the general population. The low average age of diagnosis (32 years old) and reduced life expectancy (74.7 years for pwMS compared to 81.8 years for the general population) emphasize the need for lifelong support and disease management, placing a significant financial burden on healthcare systems (Lunde et al., 2017).

The annual healthcare costs for pwMS vary by country, healthcare system type and disease severity. These costs typically include a) direct medical costs, such as medications, hospitalizations, outpatient visits, and medical procedures, b) non-medical costs, such as assistive devices, home adaptations, and informal care provided by family members or caregivers, and c) indirect costs, which encompass productivity losses due to disability, early retirement, and absenteeism (Bebo et al., 2022; Paz-Zulueta et al., 2020).

In Europe, the overall economic burden for healthcare systems treating pwMS is approximately €40,000 per patient, covering both direct healthcare and indirect costs (Paz-Zulueta et al., 2020). In the United States, the total economic burden is estimated at

$85,400 per patient, with direct medical costs accounting for $63,300 and indirect and non-medical costs reaching $22,100. The largest direct cost components include prescription medications (54%), clinic-administered drugs and administration (12%), and outpatient care (9%) (Bebo et al., 2022).

Furthermore, pwMS and their caregivers face various psychological and social challenges, which also impact their financial situations and employment status. These difficulties are compounded by social isolation, lower QoL, poor self-rated health, and reduced productivity (Kouzoupis et al., 2010; Maguire & Maguire, 2020).

1.5 Clinical Course and Subtypes of Multiple Sclerosis

To our knowledge, MS is characterized either by periods of acute relapses, either by gradually progressive worsening of the neurological functions or even combination of both. For many years, clinicians and researchers were conducting various clinical trials and discussions related to MS, but without any agreement on definitions for the various MS clinical subtypes. Therefore, the lack of such agreement highlighted the need for a reassessment of the terms used to define MS and for more constant definitions of MS subtypes (Lublin & Reingold, 1996).

In 1996, the Advisory Committee on Clinical Trials in MS, of the United States National Multiple Sclerosis Society, introduced for the first time the MS clinical subtypes, which consisted of the RRMS, the Secondary Progressive MS, the Primary Progressive MS and the Progressive Relapsing MS (Lublin & Reingold, 1996). Although, the four clinical subtypes of MS were established, the Committee in 2013 dropped the terms Progressive Relapsing MS, because it was believed to be unclear and overlapped with other disease course subtypes. Therefore, they re-examined the clinical subtypes by exploring clinical imaging and biomarker advances and reported a new disease course, the clinically isolated syndrome (Lublin et al., 2014).

1.5.1 Clinically isolated syndrome

Clinically isolated syndrome constitutes the first presentation of the disease in about 85% of pwMS (Miller et al., 2012). Clinically isolated syndrome is defined as the first clinical event of demyelinating disease, causing neurological symptoms which last for more than 24 hours. Usually symptoms are monofocal, affecting the optic nerve, spinal cord,

cerebellum and brain stem with acute progression over days to weeks (Klineova & Lublin, 2018). Although, the majority of people with clinically isolated syndrome may have a physiological brain magnetic resonance imaging (MRI), the clinically isolated syndrome can be the first sign of developing MS in the future. Several studies reported that 60% – 70% of people with clinically isolated syndrome, within 20 years they develop a second demyelinating event, as a result to be diagnosed with one of the MS phenotypes (Kuhle et al., 2015; Wottschel et al., 2015). Various MRI markers have verified prognostic potential, such as the abnormal T2 weighted image, which is associated with high risk of conversion to MS. Also, the number and volume of T2 lesions can be predictive for disability and for the early changes in whole-brain volume (Odenthal & Coulthard, 2015).

1.5.2 Relapsing-Remitting Multiple Sclerosis (RRMS)

RRMS is the most common clinical phenotype of MS, which is characterized by alternating periods of relapses and periods of relative clinical stability. Although, the annual MS relapse rate ranges from 0.27 to 1.66 relapses per year (Inusah et al., 2010) people with RRMS (pwRRMS) may experience at least one relapse every three years (Rooney et al., 2020). During the relapse period, neurological dysfunctions with full recovery or with residual deficit upon recovery are present, while in the stable period of the disease, no new neurological symptoms are presented (Figure 1.5) (Klineova & Lublin, 2018).

Figure 1.5. Disease course of Relapsing-Remitting Multiple Sclerosis.

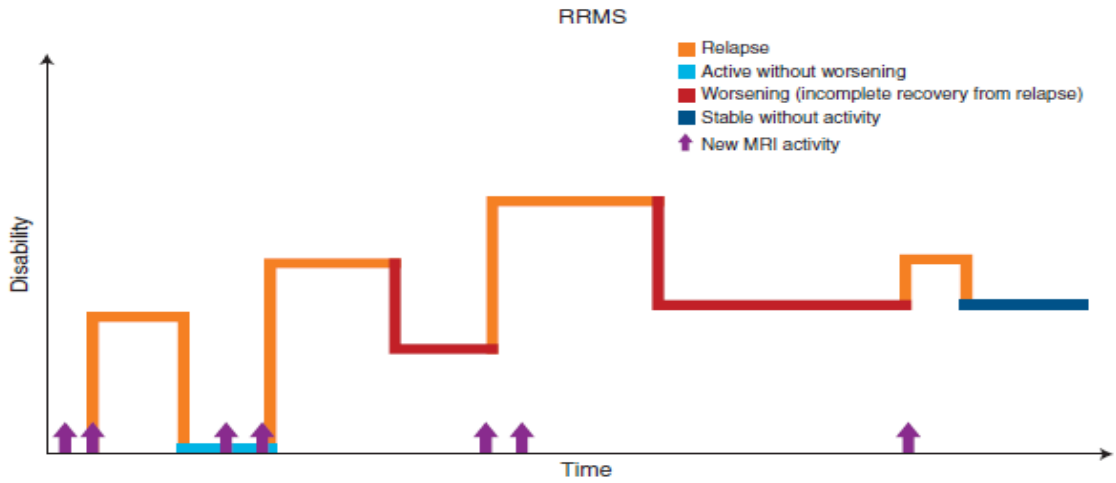


Figure 1.5: RRMS; Relapsing-remitting Multiple Sclerosis, MRI; Magnetic Resonance Image. The specific type of MS it is characterised by frequent relapses. Individual symptoms get worse

during a relapse, followed by a remission. During the remission period, individual symptoms may partly or completely go away as a result some of the symptoms to continue and become permanent. Also, it can be described as either active (relapses and/or evidence of new MRI activity) or not active, as well as worsening (increased of disability after a relapse) or not worsening (Klineova & Lublin, 2018).

Relapse episodes in MS are characterized by localized areas of inflammation, marked by perivascular lymphocytic infiltration, followed by demyelination and axonal degeneration. In the early inflammatory stages of the disease, remyelination plays a crucial role in the recovery of neurological symptoms. It is well-established that the frequency of relapses and the severity of inflammatory pathology decline with increasing age and disease progression. Several factors have been identified as contributing to an increased relapse rate, including stress, pregnancy, biochemical changes, upper respiratory infections, urinary tract infections and gastroenteritis. Moreover, the number of relapses during the early stages of MS is considered one of the strongest predictors of the time to disability onset (Klineova & Lublin, 2018).

Neurophysiological studies using Transcranial Magnetic Stimulation (TMS) have reported acute changes in corticospinal excitability and motor output reorganization following disease relapses (Chieffo et al., 2019; Wirsching et al., 2018). Chieffo et al. (2019), specifically observed bi-hemispheric alterations in corticospinal excitability after an acute motor relapse affecting one upper limb. These changes included a reduction in corticospinal output from the ipsilesional hemisphere and temporary hyperexcitability in the contralesional hemisphere (Chieffo et al., 2019).

Alterations in corticospinal plasticity are thought to reflect underlying synaptic dysfunction associated with the acute pathological processes of relapse in MS. This dysfunction may arise from inflammatory demyelination, axonal injury, or other neurophysiological disruptions occurring during relapse. Alternatively, these alterations might represent compensatory mechanisms aimed at preserving motor function and mitigating the impact of new or expanding brain lesions (Wirsching et al., 2018).

Mori et al. (2019), further elucidated the relationship between corticospinal excitability and relapse recovery outcomes in pwMS. These findings indicated that corticospinal excitability remained within normal ranges in individuals who achieved complete functional recovery following a relapse, suggesting the successful resolution of relapse-

associated pathology. In contrast, those with incomplete or absent recovery exhibited reduced corticospinal excitability, likely indicative of persistent neural damage or an insufficient compensatory response to lesions (Nisticò et al., 2014). These results underscore the critical role of corticospinal function as both a marker of disease activity and a potential predictor of recovery outcomes in MS.

RRMS is often the initial diagnosis, nearly half of relapses contribute to a stepwise accumulation of clinical impairment. Though, nearly half of the relapses lead to stepwise accrual of the clinical impairment, the MS symptoms may differ greatly from person to person and over the course of the disease, due to the location of the affected neurons. Symptoms commonly include weakness, numbness, spasticity, altered sensation, impaired balance, lack of coordination, visual deficits, cognitive dysfunctions and behavioural issues which last at least 24 hours (Akbar et al., 2020; Benedict et al., 2020; DeLuca et al., 2020; Kister et al., 2013; Klineova & Lublin, 2018; Writer & Olek, 2021). Over time, most pwRRMS transition to a progressive form, where neurological decline becomes more continuous rather than relapse-dependent. Understanding the mechanisms underlying PMS is crucial for developing effective intervention strategies to slow disease progression and improve patient outcomes.

1.5.3 Progressive Forms of Multiple Sclerosis (PMS)

PMS represents a stage of the disease characterized by a continuous worsening of neurological function, with or without superimposed relapses. PMS includes two main subtypes: Secondary Progressive MS and Primary Progressive MS. Secondary Progressive MS develops in individuals initially diagnosed with RRMS, as the disease gradually shifts from a relapsing course to a steady accumulation of disability. In contrast, Primary Progressive MS is characterized by progressive neurological decline from the onset, without distinct relapses or remissions. While inflammation plays a key role in the early stages of MS, neurodegeneration becomes a dominant feature in progressive forms, leading to irreversible damage to the CNS.

Secondary Progressive Multiple Sclerosis

Most of pwRRMS over the course of about 10 – 15 years switch to Secondary Progressive MS (Rovaris et al., 2006). A key indicator of this transition is a noticeable worsening of

baseline neurological function between relapses, signaling the shift from RRMS to Secondary Progressive MS (Cree et al., 2021).

The specific form of MS is characterized by a gradual progression of the disease, with or without the occurrence of relapses. It is more prevalent in males and is associated with several factors, including a higher age at RRMS onset, incomplete recovery from relapses and the presence of spinal cord symptoms. Common spinal cord symptoms include limb weakness, sensory loss and altered reflexes. These features underscore the progressive nature of Secondary Progressive MS and its distinct clinical profile compared to RRMS (Rovaris et al., 2006).

The primary characteristic of progressive MS phenotypes is the gradual expansion of pre-existing white matter lesions (Fischer A.J.; Kniker, J.E.; Rudick, R.A.; Cutter, G., 2001; Luchetti et al., 2018). In Secondary Progressive MS, the disease course includes periods of gradual progression, often accompanied by possible relapse activity, as well as intervals of relative stability (Figure 1.6).

Figure 1.6. Disease course of Secondary Progressive Multiple Sclerosis.

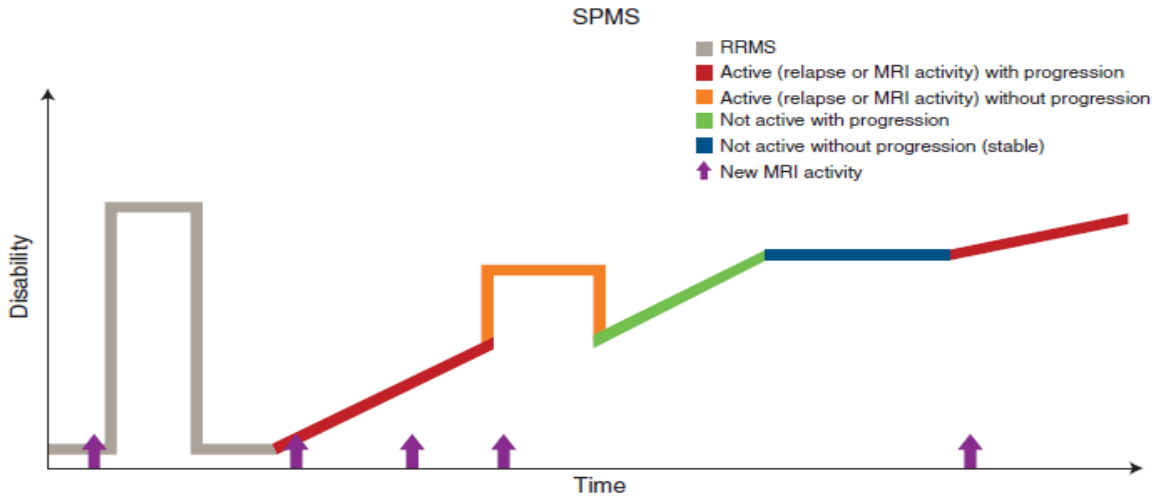


Figure 1.6: SPMS; Secondary Progressive Multiple Sclerosis, RRMS; Relapsing-remitting Multiple Sclerosis, MRI; Magnetic Resonance Image. The specific type of multiple sclerosis it is characterised by increased disability over time, followed a period of relapsing-remitting disease. It can be present with or without evidence of disease activity (relapses or changes on MRI) and may have occasional relapses or periods of stability. Also, it can be described as active (relapses and/or evidence of new MRI activity) or not active, as well as with progression (increased disability over time, with or without relapses or new MRI activity) or without progression (Klineova & Lublin, 2018).

Although CNS inflammation decreases with disease duration and advancing age (Correale & Marrodan, 2019), Secondary Progressive MS is marked by a combination of persistent inflammation and neurodegeneration. This neurodegeneration is largely attributed to mitochondrial dysfunction and subsequent axonal damage (Klineova & Lublin, 2018).

Furthermore, Correale et al. (2019), observed reduced blood-brain barrier permeability in Secondary Progressive MS compared to the acute phases of relapses in RRMS (Correale & Marrodan, 2019). Chronic lesions in Secondary Progressive MS also exhibit less severe blood-brain barrier damage compared to acute lesions. These findings explain the ongoing neurodegeneration and progressive disability characteristic of Secondary Progressive MS

Primary Progressive Multiple Sclerosis

Approximately 15% of total pwMS are diagnosed with the other progressive phenotype of the disease, known as Primary Progressive MS (Antel et al., 2012). The hallmark feature of Primary Progressive MS is a gradual and continuously worsening baseline of neurological function without distinct relapses (Lublin & Reingold, 1996). Interestingly, the progression rate in Primary Progressive MS is not influenced by age of onset or gender (Rice et al., 2013).

During the course of Primary Progressive MS, minor fluctuations may occur, including periods of apparent relapses or temporary stability (Figure 1.7). The absence of initial relapses in pwPMS may be attributed to clinically silent lesions that avoid detection during early disease stages (Klineova & Lublin, 2018). Despite the greater levels of disability observed in people with Primary Progressive MS, they exhibit fewer abnormalities on MRI compared with other MS subtypes. Over time, individuals with Primary Progressive MS tend to develop fewer new lesions than those with relapsing forms of the disease.

Figure 1.7. Disease course of Primary Progressive Multiple Sclerosis.

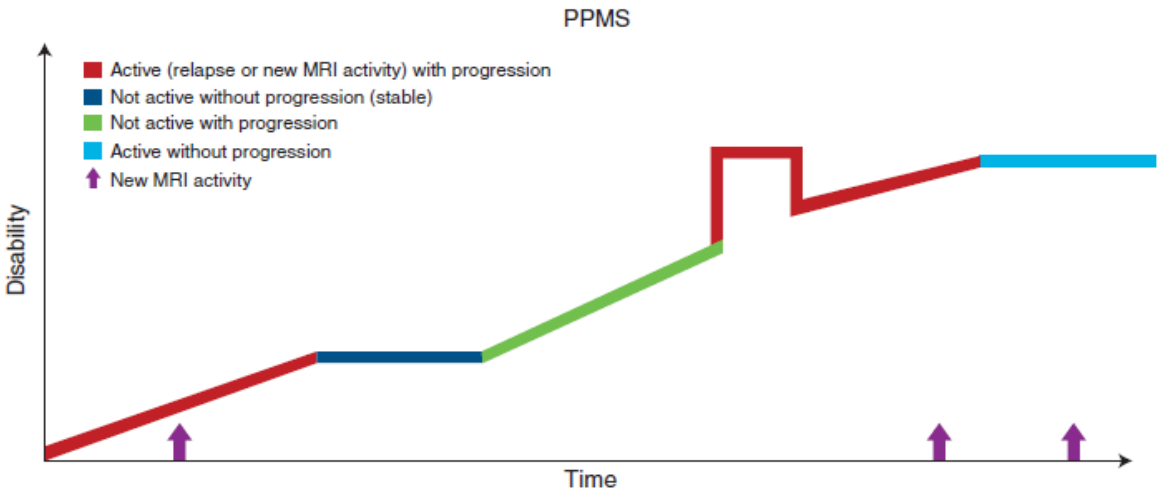


Figure 1.7: PPMS; Primary Progressive Multiple Sclerosis, MRI; Magnetic Resonance Image. The specific type of multiple sclerosis it is characterised by periods of disease stability, with or without a relapse or new MRI activity. Also, it can be presented by periods of increased disability with or without new relapses or MRI lesions. It can be described as either active (sporadic relapse and/or evidence of new MRI activity) or not active, as well as with progression (increased disability, with or without relapse or new MRI activity) or without progression (Klineova & Lublin, 2018).

To establish the diagnosis of Primary Progressive MS, evidence of intrathecal synthesis of immunoglobulin G, combined with at least one of three specific MRI criteria, is required a) nine or more brain lesions, b) two or more spinal cord lesions, or c) a combination of four to eight brain lesions and one spinal cord lesion. In cases where MRI findings alone are insufficient for diagnosis, a delayed visual evoked potential (a test measuring the electrical response of the visual cortex to visual stimulation) can be used as a supplementary diagnostic tool (Montalban, 2005).

The term "progression" refers to a continuous worsening of neurological dysfunction lasting at least 6 – 12 months. A definitive diagnosis of Primary Progressive MS requires evidence of clinical progression for a minimum of one year, along with two of the following criteria, a) MRI-detected lesions in the brain or spinal cord or b) positive cerebrospinal fluid findings, such as an increased number of white blood cells, elevated antibodies, or the presence of neurofilament light chain (Rovaris et al., 2006).

Compared to RRMS and Secondary Progressive MS, individuals with Primary Progressive MS often exhibit more diffuse brain lesions and a higher prevalence of spinal

cord lesions (Willis & Fox, 2016). Unlike the overall gender disparity in MS, where the female-to-male ratio is approximately 3:1 (Portaccio et al., 2024). Primary Progressive MS is characterized by a nearly equal gender distribution (1:1). Additionally, Primary Progressive MS typically presents at a later mean age of onset, around 40 years old, and is rarely observed in childhood (Antel et al., 2012; Rice et al., 2013).

Clinically, people with Primary Progressive MS frequently present with spastic paraparesis, cerebellar ataxia, spinal syndromes, cognitive dysfunction, and visual impairments. Disease progression in Primary Progressive MS is often marked by significant disability; within eight years of disease onset, most patients require unilateral assistance to walk, and after 18 years, many become wheelchair-dependent bound (Rice et al., 2013).

1.6 General symptoms of Multiple Sclerosis

Due to the disease pathophysiology and due to the lesion location, symptoms are highly unpredictable. MS symptoms vary among patients and within a patient over time. Some dysfunctions tend to be more dominant or have greater impact on overall disability (Ghasemi et al., 2017). The most predominant clinical symptoms in pwMS involve motor disturbances and cognitive deficits.

Motor dysfunctions commonly observed in pwMS include muscle weakness, impaired manual dexterity, gait disturbances, reduced balance and spasticity, which can affect both upper and lower limbs (Cameron & Nilsagard, 2018; Norbye et al., 2020; Wens et al., 2014). Wens et al. (2014), reported that MS alters muscle fibre characteristics, reducing muscle mass and strength regardless of disease severity (Wens et al., 2014). These motor dysfunctions significantly impair ADLs, with upper limb and manual dexterity deficits particularly problematic (Bertoni et al., 2015; Johansson et al., 2007; Lamers & Feys, 2014). Balance and gait disturbances are also prevalent, with 50% to 80% of pwMS experiencing impairments in both static and dynamic balance, as well as reduced gait speed and endurance. This increases the risk of falls (Cameron & Nilsagard, 2018). Contributing to these deficits are limited postural control and delayed responses to positional changes. Furthermore, more than 80% of pwMS develop lower limb spasticity within five years of diagnosis, leading to pain, disrupted sleep, fatigue and an increased fall risk (Norbye et al., 2020).

Furthermore, fatigue is one of the most common symptoms in MS, affecting approximately 80% of patients (Fisk, Pontefract, et al., 1994; Krupp et al., 1989; Marchesi et al., 2020). Fatigue emerges early in the disease, contributing to both cognitive and motor dysfunction, and is often associated with depressive symptoms (Diamond et al., 2008; Guillemin et al., 2022; Oervik et al., 2017; Sedaghati et al., 2023). Neuroimaging studies suggest that MS-related fatigue is linked to lesions in the frontal lobe and atrophy of the corpus callosum and basal ganglia (Langley et al., 2023; Morgante et al., 2011; Yaldizli et al., 2011). Exercisee, particularly aerobic activity combined with strength training, has been shown to reduce fatigue and improve overall function (Charvet et al., 2014; Motl et al., 2005).

Cognitive impairment is another hallmark of MS, with the prevalence and severity of cognitive deficits correlating with disease progression. Approximately 34% of individuals with Clinically Isolated Syndrome exhibit cognitive dysfunction, with the rate rising to about 50% in RRMS and reaching 80% to 90% in PMS impairment (Norbye et al., 2020; Ruano et al., 2017). Cognitive decline is linked to greater disability, longer disease duration and aging (Ruano et al., 2017). Among the various cognitive impairments, deficits in information processing speed are the most common and are recognized as one of the earliest signs of cognitive decline in PMS (DeLuca et al., 2020; Kouvatsou et al., 2020). Information processing speed refers to the brain's ability to efficiently process and respond to incoming information, whether auditory, visual, or motor-related tasks (Vernon, 1983). Slowed information processing speed affects higher-order cognitive functions, such as executive functioning, decision-making, goal-setting, and time management (Kail & Salthouse, 1994; Lindenberger & Baltes, 1994).

Studies indicate that between 40% and 70% of pwMS experience deficits in information processing speed (Oreja-Guevara et al., 2019), which have been linked to increased depression (Diamond et al., 2008; Landrø et al., 2004; Sundgren et al., 2013), fatigue (Andreasen et al., 2010; Diamond et al., 2008; Sandi et al., 2015) and reduced QoL (Eizaguirre et al., 2018; Glanz et al., 2010; Sandi et al., 2015). Information processing speed deficits worsen with disease progression, becoming more pronounced in pwPMS than in pwRRMS (Brochet et al., 2022; Giedraitienė et al., 2015; Glanz et al., 2010; Renner et al., 2020; Rosti-Otajärvi et al., 2014; Sundgren et al., 2013). However, a study by McKay et al. (2022), suggested that information processing speed deficits in RRMS

may be transient, influenced by inflammatory activity, with improvements observed after remission. In contrast, these deficits become more permanent in PMS due to neurodegenerative processes (McKay et al., 2022).

Recent research by Akaishi et al. (2024), indicated that basal ganglia atrophy is a key contributor to information processing speed deficits in MS (Akaishi et al., 2024). Significant correlations were observed between putamen and corpus callosum atrophy and impaired information processing speed in pwPMS compared to pwRRMS. Notably, no significant differences in information processing speed performance were found between individuals with Primary Progressive MS and those with Secondary Progressive MS, suggesting similar underlying mechanisms for information processing speed decline in both progressive forms of MS (Amato et al., 2010; Ukkonen et al., 2009).

These findings align with research by Manca et al. (2018), emphasizing the role of anterior corpus callosum integrity in information processing speed performance (Manca et al., 2018). The corpus callosum is also crucial for interhemispheric communication, influencing motor function and coordination. Reduced structural integrity of the corpus callosum has been linked to impaired bimanual coordination, delayed reaction times, and asymmetrical motor performance, which are evident in both RRMS and PMS. Increasing callosal atrophy correlates with deficits in gait, balance and upper limb function, impacting ADLs. Damage to the anterior corpus callosum, which connects motor-related cortical areas, can make it difficult to synchronize limb movements, further affecting ADL.

Despite the extensive literature on various clinical symptoms in MS, neurophysiological changes also occur in this clinical cohort. One of the most prominent neurophysiological-based symptoms is the disruption of corticospinal tract integrity, which plays a critical role in motor control and voluntary movement execution. Degeneration of the corticospinal tract, caused by demyelination and axonal loss, impairs motor function. TMS studies have shown alterations in several TMS-based neurophysiological measures, indicating disruption of neural transmission and structural degradation (Stampanoni Bassi, Buttari, et al., 2020). These changes are linked to motor deficits such as spasticity, reduced muscle strength, and impaired dexterity symptoms (Balloff et al., 2022; Bergsland et al., 2015; Kerbrat et al., 2020; Neva et al., 2016; Pawlitzki et al., 2017; Tovar-moll et al., 2014). Corticospinal dysfunction contributes to increased reaction

times and reduced force production, which in turn worsens gait disturbances and upper limb impairments. As corticospinal integrity declines, compensatory mechanisms involving alternative motor pathways become less effective, leading to progressive motor disability. Understanding these neurophysiological alterations is essential for developing targeted rehabilitation strategies aimed at preserving corticospinal function and mitigating motor decline in people with MS.

Living with MS presents significant challenges, as patients must cope with an unpredictable disease course marked by relapses and the potential for progression. Along with motor and cognitive impairments, pwMS often face psychological and socioeconomic difficulties that impact both their personal and professional lives (Boeschoten et al., 2017). Demyelination and neurodegeneration in MS contribute to emotional disturbances such as depression and anxiety, further reducing quality of life QoL (Kalb, 2007). Radlak et al. (2021), noted that emotional changes can worsen fatigue and impair medication adherence, attention, and concentration (Radlak et al., 2021).. The impact of MS extends to caregivers, who play a vital role in supporting patients with ADLs, medical management, and emotional care. However, the demands of caregiving can lead to stress, burnout, and reduced well-being, highlighting the importance of strong caregiver-patient relationships. These relationships can foster resilience and emphasize the need for robust support systems and resources to maintain the health and QoL of both patients and well-being (Bassi et al., 2020; Kouzoupis et al., 2010; Maguire & Maguire, 2020).

MS is a multifaceted and progressive neurological disorder that presents with a diverse range of symptoms, including motor impairments, cognitive dysfunctions fatigue and emotional disturbances, all of which significantly impact the QoL of individuals living with the disease (Benedict et al., 2020; Clough et al., 2020; Kister et al., 2013; Norbye et al., 2020). The variability of symptoms, influenced by disease course and severity, underscores the complexity of MS and the need for personalized treatment approaches to optimize functional outcomes (Biernacki et al., 2019).

Given the heterogeneity in symptom manifestation, accurate and early diagnosis is essential for effective disease management. Diagnostic tools and assessments play a crucial role in monitoring disease progression and tailoring interventions. Advanced imaging techniques, such as MRI, are instrumental in visualizing lesions and atrophy in

the CNS, aiding in the identification and monitoring of MS-related neurodegeneration. Functional assessments, including cognitive tests, motor function evaluations, and balance assessments, help clinicians understand the extent of cognitive and motor impairments, such as information processing speed deficits, spasticity and gait disturbances. Assessment tool like the Expanded Disability Status Scale (EDSS) is routinely used to assess overall disability and specific functional impairments, providing valuable information for tracking disease progression and treatment efficacy. Additionally, neurophysiological assessments, such as TMS, are increasingly utilized to evaluate corticospinal tract integrity, offering insights into motor dysfunction and facilitating the development of targeted rehabilitation strategies.

1.7 Assessment and Diagnostic Tools in Multiple Sclerosis

1.7.1 Expanded Disability Status Scale (EDSS)

The EDSS was developed by neurologist John Kurtzke in 1983 to quantify and monitor changes in the disability levels of pwMS over time. The EDSS assesses disability through a neurological examination, describing symptoms and signs across eight functional systems: pyramidal, brainstem, cerebellar, cerebral (mental), bowel and bladder, sensory, visual, and other functions (Appendix I - Functional Systems). The EDSS score ranges from 0 (indicating no disability in any functional system) to 10 (representing death due to MS) (Table 1.1) in half-point increments (Kurtzke, 1983).

Although the EDSS is widely used to assess disability progression in pwMS, several limitations affect its reliability. First, the subjective evaluations of neurologists can vary, leading to inconsistencies in clinical descriptions between physicians. Second, EDSS scores above four focus primarily on ambulatory status, with less emphasis on upper-body functions. Finally, the scale inadequately assesses cognitive function, energy levels, mood, and QoL, which are common and significant symptoms in pwMS (van Munster & Uitdehaag, 2017).

Table 1.1. Score and description of the Expanded Disability Status Scale.

Score	Description
0	No disability, normal neurological exam.
1.0	No disability, minimal signs in one functional system.
1.5	No disability, minimal signs in more than one functional system.
2.0	Minimal disability in one functional system.
2.5	Mild disability in one functional system or minimal disability in two functional system.
3.0	Moderate disability in one functional system, or mild disability in three or four functional systems. No gait impairment.
3.5	Moderate disability in one functional system and more than minimal disability in several others. No gait impairment.
4.0	Significant disability but self-sufficient and up and about some 12 hours a day. Able to walk without aid or rest for 500m.
4.5	Significant disability, but able to work a full day. May have some limitation of full activity or require minimal assistance. Able to walk without aid or rest for 300m.
5.0	Severe disability. Full daily activities and ability to work a full day without special provisions. Able to walk without aid or rest for 200m.
5.5	Severe disability. Preclude full daily activities. Able to walk without aid or rest for 100m.
6.0	Requires a walking aid to walk about 100m with or without resting.
6.5	Requires two walking aids to walk about 20m without resting.
7.0	Unable to walk beyond 5m (with or without walking aid). Essentially restricted to wheelchair (12 hours a day), transferring alone.
7.5	Unable to take more than a few steps. Restricted to wheelchair and may need aid in transferring. May require a motorised wheelchair

8.0	Essentially restricted to bed or chair. May be out of bed itself much of the day. Retains many self-care functions. Generally, has effective use of arms.
9.0	Confined to bed. Can still communicate and eat.
9.5	Totally dependent. Confined to bed and unable to communicate effectively or eat/swallow.
10.0	Death due to MS

Table 1.1: MS; Multiple Sclerosis. The Expanded Disability Status Scale it is used to quantifying disability in pwMS over time, which is rated from 0 (no disability) to 10 (death) in 0.5 unit (Kurtzke, 1983).

1.7.2 Diagnostic tools (Clinical Examination, Lumbar Puncture, Blood Tests, MRI, Neurophysiological Examinations)

The diagnosis of MS can be made when there is evidence of at least two distinct MS attacks. The fundamental diagnostic tools include clinical examination, lumbar puncture, blood tests, MRI and neurophysiological examinations, which collectively establish a baseline profile (Dobson & Giovannoni, 2019). The neurologist conducts a thorough clinical examination, often described using the EDSS. This examination evaluates alterations in movement patterns, potential weakness or spasticity, changes in balance, discoordination, abnormal reflexes, cognitive dysfunction and visual and speech deficits (Sharrack & Hughes, 1996).

A lumbar puncture is a key diagnostic procedure that involves collecting cerebrospinal fluid to investigate potential biomarkers associated with MS. This procedure can detect elevated levels of immunoglobulin antibodies and oligoclonal bands, immune system proteins that indicate myelin damage, which are not present in the blood. Blood tests are also recommended to screen for conditions that may mimic MS or influence its progression. These include assessments of vitamin B12 levels, thyroid function and serological tests for syphilis, human immunodeficiency virus 1 serology, human T-cell lymphotropic virus 1 and 2 serology and anti-myelin oligodendrocyte glycoprotein antibody (Dobson & Giovannoni, 2019; Palace, 2001).

MRI is a valuable diagnostic tool for confirming clinically isolated syndrome (Sahraian & Eshaghi, 2010) and diagnosing MS using T2-weighted images. It enables the

evaluation of brain and/or spinal cord lesions in terms of both time and space, as well as brain atrophy (Calabrese et al., 2010; Filippi et al., 2016; Filippi & Rocca, 2011). Brain lesions observed via MRI vary in size (e.g., 3 – 6 mm) and shape (e.g., homogeneous nodular or ring-shaped), depending on the degree of blood-brain barrier breakdown and the severity of inflammation (Filippi et al., 2019). Spinal cord lesions are typically located dorsolaterally and are shorter than two vertebral body segments in length (Figure 1.8). Brain and spinal cord atrophy observed in pwMS via MRI reflects neurodegeneration and axonal loss at all stages of the disease (Sahraian & Eshaghi, 2010).

Figure 1.8. Brain and spinal cord MRI examples of lesion locations in a patient with Multiple Sclerosis.

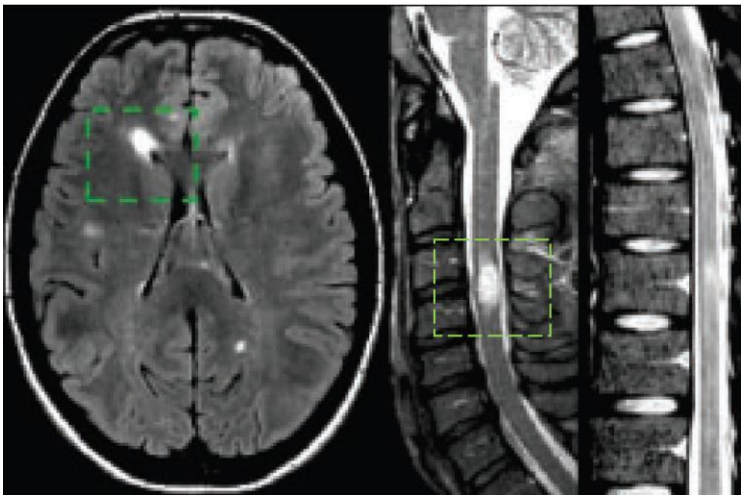


Figure 1.8: Multiple Sclerosis lesions appear on an MRI scan as either bright or dark spots and tend to have an oval or frame shape (Filippi et al., 2019).

Interestingly, the estimated rate of brain atrophy in pwMS is 0.6% – 1.35% per year, with the highest rates observed in patients with active RRMS (Bermel & Bakshi, 2006). In contrast, abnormalities in brain MRI are often less pronounced in PMS due to the prevalence of spinal cord lesions and/or a different pathological spectrum, such as generalized axonopathy (Palace, 2001).

MRI plays a crucial role in applying the McDonald criteria for diagnosing MS. By providing specific evidence of lesion dissemination in both time and space, MRI enables earlier diagnosis, particularly in patients with clinically isolated syndrome (Tomassini, Matthews, et al., 2012). The McDonald criteria set clear requirements for confirming an MS diagnosis, making MRI an essential tool in the diagnostic process (Table 1.2) (Writer & Olek, 2021).

Table 1.2. Diagnostic criteria for Relapsing-Remitting Multiple Sclerosis and Primary Progressive Multiple Sclerosis.

MacDonald 2017, criteria for Relapsing-Remitting Multiple Sclerosis	
Dissemination in space	Either (i) Objective clinical evidence of ≥ two lesions or objective clinical evidence of one lesion, with reasonable historical evidence of a prior attack involving a different CNS site or (ii) ≥ one T2 lesion in at least two out of four MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, spinal cord).
Dissemination in time	Either (i) ≥ two attacks separated by at least one month or (ii) Simultaneous presence of asymptomatic gadolinium enhancing and non-enhancing lesions at any time or (iii) A new T2 and/or gadolinium-enhancing lesion on follow-up MRI irrespective of its timing, with reference to a baseline scan or (iv) Demonstration of CSF-specific oligoclonal bands (as a substitute for dissemination in time).
MacDonald 2010, criteria for Primary Progressive Multiple Sclerosis.	
(i) One year of disease progression (retrospectively or prospectively determined) and (ii) Two out of three of: a) evidence of dissemination in time in the brain based on ≥1 T2 lesion in at least one area characteristic for MS (periventricular, juxtacortical, infratentorial). b) evidence of dissemination in time in the spinal cord based on ≥2 T2 lesions	

c) positive CSF (oligoclonal bands on isoelectric focusing and/or elevated immunoglobulin G index).

Table 1.2: CNS; Central Nervous System, MS; Multiple Sclerosis, MRI; Magnetic Resonance Image, CSF; Cerebrospinal Fluid. The McDonald criteria for people with MS include clinical, laboratory and radiographic measures which are used in the MS diagnosis.

Notable, there is an important relationship between MRI findings and individual clinical status, known as the "**clinicoradiological paradox**". This dissociation is explained by the fact that MRI-detected lesions are often clinically silent and by the limitations of the EDSS described earlier. As a result, MRI findings may not always correlate with clinical disability (Goodin, 2006; Skorić et al., 2014). However, T2-weighted alterations have been shown to have a stronger relationship with cognitive dysfunction than with physical disability (Rovaris et al., 1998). MRI provides data associated with anatomical structures, while neurophysiological examinations, including evoked potentials, offer insights into functional systems (e.g., optic nerve dysfunction, motor impairment) (Comi et al., 1993). Evoked potentials have therefore been proposed as a valuable diagnostic tool for detecting silent lesions and supporting the relationship between MS and clinical symptoms. The most commonly used evoked potentials are visual evoked potentials and motor evoked potentials (MEPs) (Habek et al., 2017).

Visual evoked potentials are part of the MS diagnostic criteria (Filippi et al., 2016) and are frequently used to evaluate patients with suspected optic neuritis, where delayed latency and reduced amplitude are observed (Chirapapaisan et al., 2015). MEPs, on the other hand, are particularly valuable for assessing the integrity of the ***corticospinal tract*** and for identifying ***neuroplastic changes*** in the brain and spinal cord via TMS (Bestmann & Krakauer, 2015; Magnano et al., 2014; Neva et al., 2016; Pascual-Leone A et al., 1998). Alterations in MEPs are commonly observed in pwMS and are correlated with EDSS scores (Fuhr et al., 2001).

1.8 Neurophysiological Basis of Motor Function and Plasticity

1.8.1 Corticospinal Tract: Anatomy, Function, and Role in Motor Control

The corticospinal tract is one of the major white matter motor pathways and the largest descending tract of the spinal cord. It originates from pyramid-shaped cells in the

premotor, primary motor and primary sensory cortices and is responsible for controlling voluntary motor activity (Barthélemy et al., 2011; Lemon, 2008; Martin, 2005). As the corticospinal tract descends into the medulla, approximately 90% of its axons cross to the contralateral side at the pyramidal decussation. The remaining 10% do not cross and continue their path within the anterior corticospinal tract, projecting predominantly to proximal and axial musculature (Figure 1.9) (Kuypers, 1982; Lacroix et al., 2004). After exiting the brainstem and entering the spinal cord, the fibres travel through the anterior and lateral corticospinal tracts. At their target spinal levels, the fibres of the anterior corticospinal tract cross to the opposite side via the anterior white commissure before synapsing with neurons in the anterior horn of the grey matter.

In contrast, the fibres of the lateral corticospinal tract, which have already crossed at the pyramidal decussation, synapse directly with neurons in the anterior horn at their respective spinal levels. The anterior corticospinal tract primarily governs proximal and axial muscle groups, playing a crucial role in postural control, whereas the lateral corticospinal tract is primarily responsible for the fine motor control of distal limb muscles. The axons of the corticospinal tract terminate at various levels of the spinal cord, synapsing with interneurons or directly with alpha motor neurons in the anterior horn, which innervate skeletal muscles (Welniarz et al., 2015). The uncrossed axons of the corticospinal tract, particularly within the anterior pathway, are essential for supporting bilateral symmetrical movements of both the upper and lower limbs (Cauraugh & Summers, 2005).

Figure 1.9. The corticospinal tract.

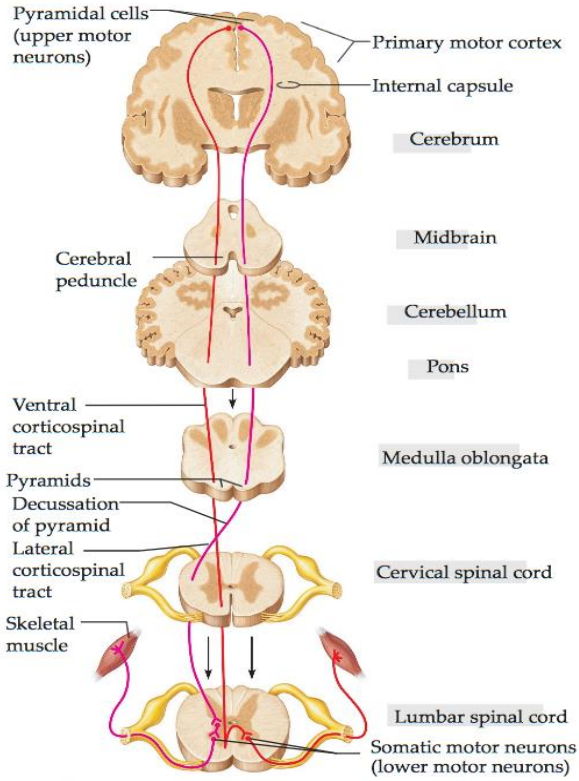


Figure 1.9: The corticospinal tract. Approximately 90%, cross the midline in the brain stem and then descend over the spinal cord in the lateral corticospinal tract (Kuypers, 1982).

The integrity of the corticospinal tract is essential for normal motor function, as it governs both fine motor control and postural stability through its lateral and anterior pathways. Damage to this critical motor pathway, such as in stroke, spinal cord injury, or demyelinating diseases like MS, often leads to significant motor deficits, including spasticity, weakness and impaired coordination. These deficits arise from the disruption of the corticospinal tract's precise neuronal connections, which are crucial for transmitting signals between the brain and spinal cord (Kauv et al., 2024; Paul et al., 2023; Pawlitzki et al., 2017).

Despite its vulnerability, the corticospinal tract exhibits remarkable adaptability following injury. Mechanisms such as axonal sprouting, synaptic reorganization and the recruitment of ipsilateral or alternate motor pathways enable varying degrees of motor recovery. This plasticity highlights the potential for rehabilitation strategies to harness the corticospinal tract's capacity for reorganization, promoting functional recovery even in the presence of structural damage (Balloff et al., 2023; Chen M et al., 2023; Liu et al., 2021).

Understanding the role of the corticospinal tract in both healthy individuals and clinical cohorts is pivotal for developing targeted therapies aimed at restoring motor function after injury or disease-induced disruption. By integrating knowledge of the corticospinal tract's anatomical pathways, functional roles and adaptive mechanisms, future research can guide innovative approaches to motor rehabilitation and improve outcomes for individuals with corticospinal tract -related impairments.

1.9 Neuroplasticity: Definition, Mechanism, Factors Influencing It

A healthy brain adapts to daily challenges and environmental changes more rapidly than than would be possible through genetic or epigenetic alterations (Pascual-Leone et al., 2005). This adaptability, known as ***"neuroplasticity"***, persists throughout life and supports essential processes such as learning, skill acquisition, memory formation and recovery from brain injury (Ksiazek-Winiarek et al., 2015).

Neuroplasticity is defined as the structural or functional changes occurring within individual neurons or across populations of neurons, such as synaptic strengthening or pruning, dendritic growth and alterations in neural pathways (Table 1.3) (Warraich & Kleim, 2010).

Understanding neuroplasticity provides insights into treatments for neurological disorders and strategies for cognitive enhancement, highlighting the brain's dynamic and resilient nature.

Table 1.3. Categories of neuroplasticity.

	Individual Neurons	**Population of Neurons**
Structural	Dendritic arbor Spine density Synapse number Synapse size Axonal arbor Receptor density	Structure thickness Gray matter density
Functional	Excitatory postsynaptic potential	Sensory map

	Neural activity Intrinsic excitability	Motor map EEG fMRI Positron emission tomography Magneto-encephalography MEPs Excitatory postsynaptic potential

Table 1.3: EEG; electroencephalography, fMRI; functional magnetic resonance imaging, MEPs; motor-evoked potentials. Structural/functional changes of the neuron or across populations of neurons, in categories of neuroplasticity (Warraich & Kleim, 2010).

By the term neuroplasticity many changes are involved, such as the formation of new synapses, alteration of the synaptic transmission strength and cortical reorganization (Ksiazek-Winiarek et al., 2015). Synapses are considering to be the main factor of the neuroplasticity process causing brain behaviour changes. The process of synaptic transmission involves the coordinated opening of ion channels and the movement of synaptic vesicles in order to release several neurotransmitters into the synaptic cleft, so they can bind to postsynaptic receptors. The postsynaptic receptors then signal changes in the postsynaptic neuron, which influence whether the specific neuron will increase or decrease its activity, causing alteration in neuroplasticity (Warraich & Kleim, 2010).

Plasticity is critical to the development of brain networks, it can assist to new skills acquisition and adaptation after a brain injury and it can be beneficial for the management of several clinical symptoms too. While the adaptive nature of neuroplasticity offers significant benefits, its efficacy can be adversely influenced by several intrinsic and extrinsic factors. These include age-related decline, genetic predispositions, and environmental conditions, all of which may diminish the capacity for neural adaptation and repair, potentially resulting in motor dysfunctions and cognitive impairments (Catarina et al., 2011; Lindenberger et al., 2008; Marzola et al., 2023).

Age is a particularly prominent factor. Plasticity of the brain generally declines with advancing age due to reductions in neurogenesis, synaptic density and cellular energy metabolism. These changes limit the brain’s ability to restructure its neural networks

effectively. Genetic factors, such as polymorphisms in genes related to neurotrophic factors (e.g., brain-derived neurotrophic factor) or synaptic proteins, can also modulate the extent of neuroplastic changes. For instance, individuals with genetic variations that reduce the availability or function of neurotrophic factors may exhibit less robust neural adaptation (Catarina et al., 2011; Lindenberger et al., 2008; Marzola et al., 2023).

Environmental factors such as chronic stress, sedentary lifestyles and poor nutrition can significantly impair neuroplasticity by disrupting hormonal balance, interfering with neurochemical signaling and weakening the structural integrity of neural networks. In contrast, enriching environments that incorporate physical exercise, mental stimulation, and social interaction have been shown to enhance neuroplasticity, highlighting the dynamic relationship between brain adaptability and external conditions (Han et al., 2023; Mishra et al., 2020). To better understand and assess neuroplasticity across different life stages and neurological disorders, various techniques, including TMS, have been developed and applied (Kricheldorff et al., 2022).

1.10 Transcranial Magnetic Stimulation (TMS) as a Tool to Assess Corticospinal Plasticity

TMS is a safe and non-invasive brain stimulation method for probing the human brain, serving as a powerful tool to induce, measure, and modulate neuroplasticity (Rossi et al., 2009). Based on the principle of electromagnetic induction, TMS allows researchers to explore brain-behavior relationships, map sensory, motor and higher-order cognitive functions (Fisher, 2007; McNeil et al., 2013) and assess the excitability, connectivity, and plasticity of cortical regions (Catarina et al., 2011).

Extensive research has demonstrated that neuroplasticity can be effectively investigated using TMS (Brasil-Neto et al., 1993; Pascual-Leone A et al., 1998). As a well-established diagnostic and therapeutic tool in clinical practice, TMS provides valuable insights into individual cortical activity. It facilitates the exploration of the pathophysiology of various neurological and psychiatric disorders by examining cortico-cortical and corticospinal connectivity. Moreover, TMS enables the identification of causal relationships between behavior and brain activity, making it a crucial technique in neuroscience research and clinical applications (Bashir et al., 2010; Pascual-Leone et al., 2005).

An electromagnetic induction, using a cooper wire coil, it is connected to a magnetic stimulator (Figure 1.10). Through the TMS procedure, the electric current it is converted to magnetic field pulses, which are delivered into the skull, parallel to the brain's cortical surface (when the coil is held tangentially to the scalp). These magnetic pulses cause electric currents in the targeted brain regions and affecting depolarization of neurons.

TMS effect can be hypothesized as the result of interactions between the induced current and the targeted brain tissues (Wagner et al., 2007). A variety of coil types, differing in shape and size, have been developed, each generating distinct magnetic fields. The geometry of a given coil determines the focality, strength and shape of the induced electric field, which in turn influences the characteristics and precision of brain stimulation.

Figure 1.10. Transcranial Magnetic Stimulation device.

Figure 1.10: Two magnetic stimulators in which a figure-eight coil it is connected (picture retrieved from https://www.ebme.co.uk/articles/clinical-ngineering/transcranial-magnetic-stimulation-of-the-brain).

The most common and frequently used types of TMS coils are the circular or round coil, the figure-eight coil, the double cone coil and the H coil (Figure 1.11). The *"circular or round"* coil is the oldest and simplest one, in which a perpendicular non-focal magnetic field occurs in the middle and it is mainly used for single pulses and peripheral stimulation (Yang et al., 2010). The *"figure-eight"* (butterfly) coil consists by two single circular coils against one another, causing a stronger and a more focal magnetic field, which is often preferred for focal stimulation of peripheral nerves (Ueno et al., 2021). The

"double-cone" coil formed from two large circular arms, which conforms to the head shape and it has the most focal magnetic field that reaches deep brain areas (Deng et al., 2014). Lastly, the *"H-Coil"* generates summation of the electric field, so to stimulate deep cortical layers at depth of 4cm – 6cm (Roth et al., 2002).

Figure 1.11. Types of Transcranial Magnetic Stimulation coils and the corresponding electric field distribution in the brain.

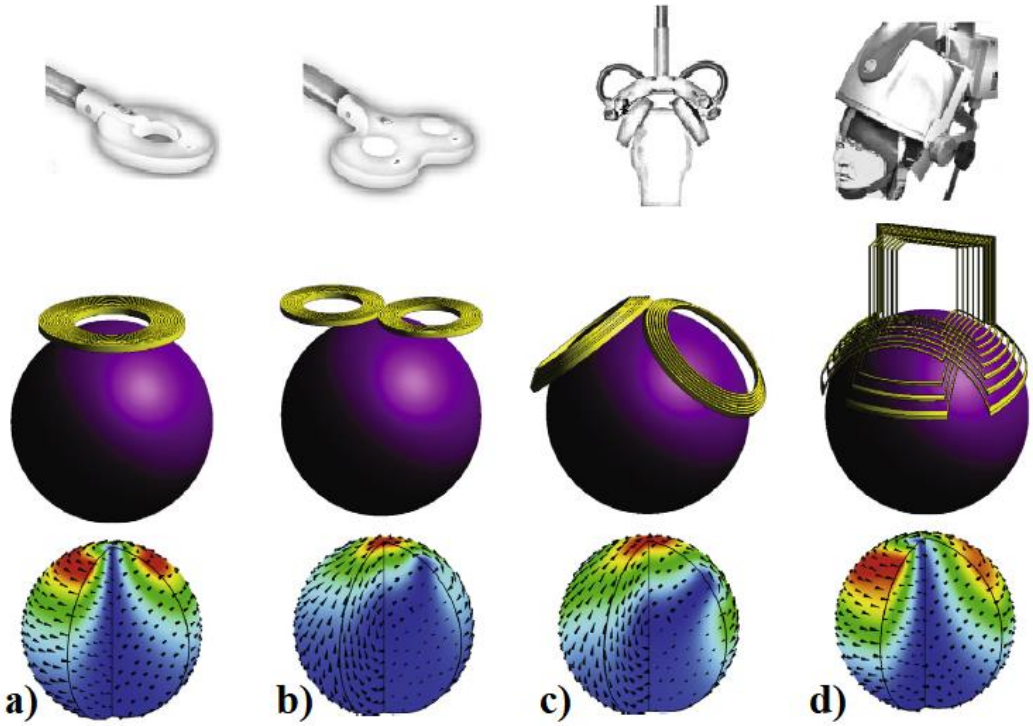


Figure 1.11: A number of different types of coils exist, each of which produces different magnetic fields. a) Circular or round coil, b) Figure-eight coil, c) Double cone coil, d) H-coil (Deng et al., 2014).

Different pulse patterns and durations, such as single-pulse, paired pulse and repetitive stimulation, can be used in a variety of clinical and academic research studies. "Single-pulse" TMS it is used for diagnostic and exploratory purposes, with the uses of isolated and modulated pulses to specific cortical areas. "Paired-pulse" TMS uses two isolated pulses, which are delivered in close sequence and it can be applied at the same or separate brain regions in order to assess functional connectivity. Finally, during the repetitive stimulation trains of pulses are delivered, in which clinicians and researchers they are using it to prompt long lasting effects in cortical reactivity, functioning and neuroplasticity (Zewdie & Kirton, 2016).

To date, brain circuitries' integrity can be measured with the use of single-pulse TMS over the primary motor cortex (M1). A series of descending corticospinal waves are produced, that they can depolarize alpha motor neurons in the spinal segmental level and lead to muscle contraction of the contralateral side. The specific muscle contraction it is defined as MEP, measured by the use of an electromyography (EMG) (Figure 1.12). (Catarina et al., 2011).

Figure 1.12. Single-pulse Transcranial Magnetic Stimulation applied over the primary motor cortex.

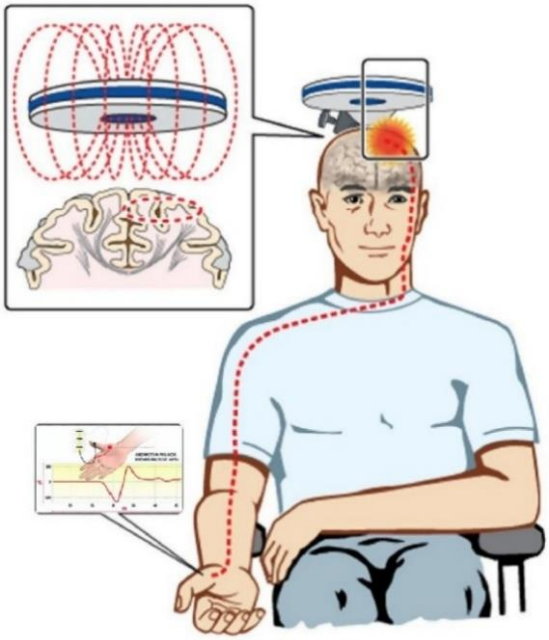


Figure 1.12: A circular coil it used to perform single-pulse Transcranial Magnetic Stimulation over the primary motor cortex of the left brain hemisphere. During the stimulation, a contraction of the targeted muscle (i.e. Abductor Pollicis Brevis), of the controlateral side it is produced, in which several outcome measures can be recorder throughout an electromyography. Picture retrieved from (Vucic et al., 2013).

Throughout the literature, TMS has been utilized to evaluate the functionality of the corticospinal tract and the corpus callosum, as well as to explore various physiological properties of the M1. Different TMS paradigms have been developed to investigate corticospinal excitability, examine excitatory and inhibitory interactions within M1, and assess M1 connectivity. Single-pulse TMS, in particular, is used to measure corticospinal plasticity (Neva et al., 2016; Pascual-Leone A et al., 1998).

1.11 Corticospinal Plasticity: Measures and Significance

Several TMS studies in both healthy and clinical cohorts have used EMG outcome measures to investigate the neurophysiology of the motor system, particularly the neuroplasticity of the corticospinal tract, a phenomenon known as "***corticospinal plasticity"*** (Bashir et al., 2010; List et al., 2013; Pascual-Leone A et al., 1998). Corticospinal plasticity refers to the ability of the corticospinal tract—the neural pathway that transmits motor signals from the brain's primary motor cortex to the spinal cord—to adapt and reorganize in response to various stimuli, injuries, or changes in motor demands. This ability is central to understanding how the nervous system responds to neurological disorders such as stroke, spinal cord injury MS. The corticospinal tract is critical for voluntary motor control, and its plasticity is essential for both recovery of function following injury and the enhancement of motor learning.

Corticospinal plasticity can be probed using single-pulse TMS (Neva et al., 2016; Pascual-Leone A et al., 1998) and described via different TMS-specific neurophysiological measures including MEP amplitude and latency, resting motor threshold (rMT) (Udupa & Chen, 2013; Ziemann, 2013) and Central Motor Conduction Time (CMCT) (Snow et al., 2019). By examining changes in MEPs amplitude and latency, insights into the plasticity of the corticospinal tract could be indicated. Increased corticospinal excitability, often referred to as "cortical hyperexcitability," is associated with positive changes in motor performance and can be induced by motor training or rehabilitation interventions. Conversely, decreased corticospinal excitability can indicate motor deficits and may reflect maladaptive changes or damage to the motor pathways.

MEPs are the electrical signals, which are recorded from the descending motor pathways of a muscle contraction, that is produced after the stimulation of the corresponding M1 area. Several outcome measures can be identified from a MEP record, including MEP amplitude and MEP latency measuring corticospinal excitability (Figure 1.13). The MEP amplitude is measured from the negative peak to the positive peak, referred to as the peak-to-peak amplitude and is expressed in units of microvolts (μV) or millivolts (mV). Changes in MEP amplitude can be influenced by excitability variations at the cortical level, making it an important marker of synaptic activity in the motor cortex (Ferreri et al., 2014). MEP latency it is defined as the time between the onset of a single-pulse TMS

and the presence of a MEP at the periphery, which characterize the integrity of the white matter fibres (i.e., diameter and myelin sheath thickness). MEP latency it is frequently used to measure the speed of central and spinal conduction time, it represented in units of milliseconds (ms) and differs through muscle location. To note, muscles of the upper limbs (e.g., biceps brachii) have shorter latency than the muscles of the lower limbs (e.g., gastrocnemius), due to the distance from the brain (Bashir et al., 2010; Day et al., 1989).

Figure 1.13. Motor evoked potential response after a single-pulse Transcranial Magnetic Stimulation.

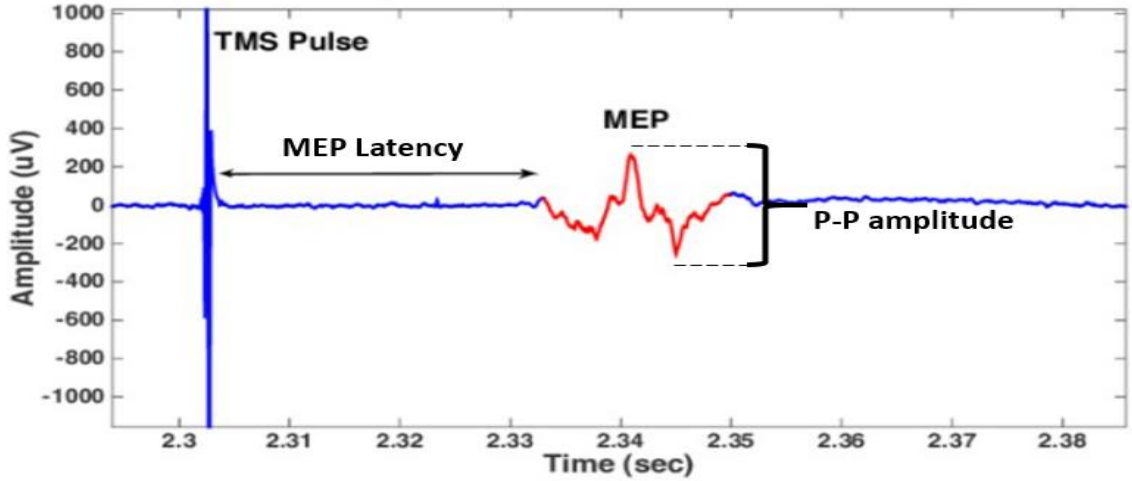


Figure 1.13: TMS; Transcranial Magnetic Stimulation, MEP; Motor Evoked Potential, P-P; peak-to-peak. When a single-pulse Transcranial Magnetic Stimulation is performed over the primary motor cortex, a muscle contraction is performed from which several electromyography-based measures can be recorded. Picture retrieved from (Marzbani et al., 2016).

Corticospinal excitability is designated throughout measuring of the motor threshold by means of TMS (Abbruzzese & Trompetto, 2002) and expressed by percentage of maximum stimulator output (%MSO). Motor threshold it is used to evaluate corticospinal tract integrity and the individual level of excitability (Bashir et al., 2010). Motor threshold can be described as a rMT in which the target muscle is at a rest state, or as an active motor threshold in which the target muscle performs a voluntary contraction. rMT is defined as the lowest TMS intensity required to induce MEPs in a target muscle of more than 50mV peak-to-peak amplitude, in at least 50% of 10 consecutive trials, when single-pulses of TMS are performed over the M1 (Rossini et al., 2015). Therefore, when the individual cortical excitability increases, the rMT reduces and vice versa (Snow et al., 2019).

CMCT represents the time which is needed for neural impulses to travel via CNS to the target muscle. During single-pulse TMS over the M1, CMCT is defined as the time required to activate cortical motor neurons via the corticospinal tract. It serves as a valuable tool for assessing the integrity of fast-conducting motor pathways within the corticospinal tract (Rossini et al., 2015). The physiological CMCT value (mean ±3 standard deviation (SD)) for the upper limb is 7.7ms, whereas for the lower limb is prolonged (i.e., 17.1ms) (Nardone et al., 2010). To our knowledge, CMCT can be calculated by subtracting the Peripheral Motor Conduction Time from the MEP latency which is provoked from a single-pulse TMS (Figure 1.14). Peripheral Motor Conduction Time it is calculated by two ways; The first way is by using the formula *(F wave latency + M wave latency -1)/2*. *F*-wave refers to the response of the target muscle, which is produced by the activation of the α-motoneuron by the antidromic volley and *M*-wave refers to the direct muscle response. Number 1 in the formula, represents the millisecond that is needed for the stimulus to turnaround over the cell body of spinal motoneuron. The whole formula is divided by two, since the latencies denote the time needed for the nerve impulse to cover the distance between the target muscle, to reach the spinal cord and return back to the target muscle. The second way, is the direct measure of latencies via stimulation of the spine that activates the nerve roots in the intervertebral foramina. However, this technique it is a quiet error because it failures to count the part form the spinal cord to the foraminal region (Hallett, 2007; Udupa & Chen, 2013).

Figure 1.14. Schematic demonstration of Central Motor Conduction Time.

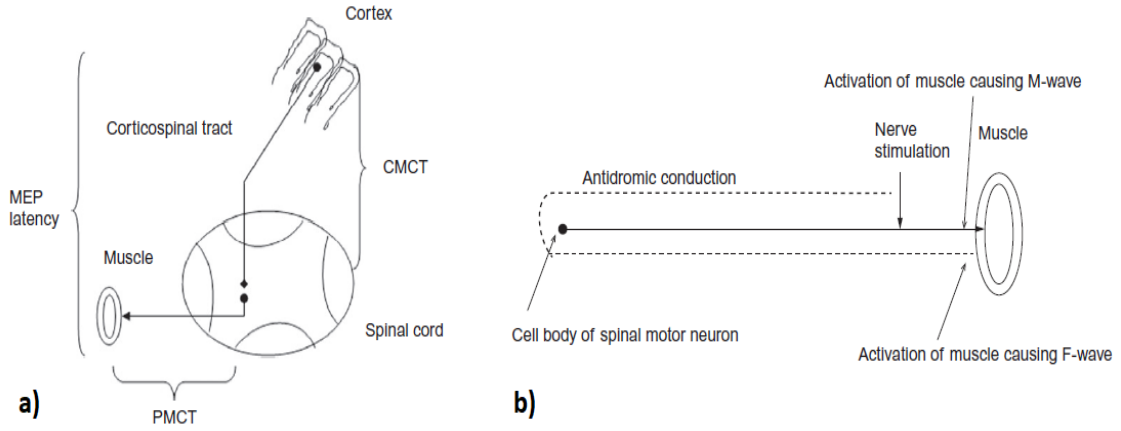


Figure 1.14: MEP; Motor Evoked Potential, CMCT; Central Motor Conduction Time, PMCT; Peripheral Motor Conduction Time. a) Central Motor Conduction Time is calculated by subtracting the PMCT from the MEP latency elicited by stimulation of the corresponding primary

motor cortex. b) Calculation of PMCT using the *F*-wave formula. The activation of the α-motoneuron by the antidromic volley elicits the *F*-wave. The latency refers to the time needed for the nerve impulse to reach the spinal cord and then return back to the target muscle (Udupa & Chen, 2013).

As it is previously described cortical dysfunctions have been demonstrated to be key components of MS condition and plasticity of the corticospinal tract is highly involved in major MS symptoms. Accordingly, corticospinal tract dysfunction in MS can be studied with neurophysiological tools, such as TMS. The specific technique is widely used to provide essential elements of MS diagnosis and can also be used to investigate plasticity changes over different treatments and interventions (Snow et al., 2019, 2024; Vucic et al., 2023).

1.12 Corticospinal Plasticity in People with Multiple Sclerosis

Throughout the literature, neuroplasticity in MS appeared to be abnormal both in RRMS and PMS (Baione et al., 2020; Ksiazek-Winiarek et al., 2015; Neva et al., 2016; Nisticò et al., 2014). MRI and fMRI studies reported that pwMS have greater bilateral motor activation than healthy controls (Reddy et al., 2002; Zeller & Classen, 2014), while various neuroplastic changes present locally at the injured area or it can be present distally over uninjured brain pathways, which explain the phenomenon of clinicoradiological paradox (Ksiazek-Winiarek et al., 2015). Neuroplasticity has a key role for the recovery from MS lesions throughout different phases of the disease, including acute relapse, chronic inflammation and axonal degeneration, which can be promoted via certain rehabilitation approaches (Dayan & Cohen, 2011).

Importantly, the *reserve* of neuroplastic potential is essential in influencing the disease course, even in the presence of widespread demyelination in white matter and neurodegeneration in grey matter, starting from the early stages of MS (Mori et al., 2013). The concept of reserve has been introduced to explain individual differences in clinical outcomes, cognitive performance and functional capacity despite similar levels of brain pathology. *Cognitive reserve* refers to the adaptive capacity of cognitive processes, which allows certain individuals to better compensate for structural brain damage or neurodegeneration. Cognitive reserve may underlie why individuals with comparable lesion burden can exhibit markedly different levels of cognitive impairment or functional

decline. In contrast, *brain reserve* is defined as the available neurobiological capital (i.e., brain volume, neuronal density, synaptic integrity), which provides a structural buffer against pathology. Brain reserve is generally considered a static trait at any given point but may be shaped over time by enriching life experiences. Additionally, the reduced accumulation of age-related or pathological changes in brain structure and function refers to the *brain maintenance*. This concept reflects the brain's modifiable nature, influenced by both genetic and lifestyle factors such as physical activity, education and cognitive engagement. Together, cognitive reserve, brain reserve and brain maintenance offer a unified framework for understanding individual variability in MS progression and recovery potential (John et al., 2024; Stein et al., 2023; Sumowski, 2015; Sumowski et al., 2014).

PwMS experience alter corticospinal tract integrity, which is associated with various clinical symptoms (Balloff et al., 2022; Bergsland et al., 2015; Kerbrat et al., 2020; Neva et al., 2016; Pawlitzki et al., 2017; Tovar-moll et al., 2014), such as general muscle weakness (Zackowski et al., 2008), decrease gait pattern and motor control of lower limbs (Fritz et al., 2017; Strik et al., 2021) and balance disturbances (Capone et al., 2019). Previous TMS-based studies in this clinical population (Ziemann, 2013), have indicated changes in corticospinal plasticity. These changes are associated with a reduction in MEP amplitude, abnormalities in rMT and MEP latency, and a prolongation of CMCT (Chalah et al., 2021; Hayward et al., 2017; Leocani, 2015; Schmierer et al., 2002; Zipser et al., 2018).

These findings suggest a combination of demyelinating conduction blocks and axonal damage as key contributors to neurological impairments in MS. Demyelination and conduction blocks can cause greater temporal dispersion of corticospinal volleys, leading to reduced MEP amplitude, increased MEP duration and prolonged MEP latency and CMCT. In contrast, axonal loss appears to play a more significant role in PMS, characterized by higher rMT, reduced MEP amplitude and prolonged CMCT (Stampanoni Bassi, Buttari, et al., 2020). TMS abnormalities have been associated with both demyelination and neuronal degeneration in various MS phenotypes. For example, prolonged CMCT and reduced MEP amplitude may indicate axonal loss or extreme asynchrony of descending volleys to spinal motoneurons caused by conduction blocks in the myelinated fibers of the corticospinal tract (Hess et al., 1986, 1987).

Experimental studies in animal models and pwMS highlighted the critical role of inflammation in disrupting synaptic function (Centonze et al., 2009). Neurophysiological abnormalities have been observed even without macroscopic damage, suggesting additional pathological mechanisms (Zeis et al., 2008). Furthermore, proinflammatory and anti-inflammatory molecules are known to influence cortical excitability in MS, contributing to synaptic dysfunction (Stampanoni Bassi et al., 2017).

1.13 Exercise, Interlimb Coordination and Neuroplasticity

1.13.1 Exercise and its Impact on Neuroplasticity in Multiple Sclerosis

Despite the increasing burden of MS, several studies reported the impact of corticospinal plasticity in functional recovery of pwMS, throughout different types of exercise and rehabilitation approaches (Flachenecker, 2015; Prosperini & Filippo, 2019; Reddy et al., 2002; Sandroff et al., 2020; Tavazzi et al., 2021; Tomassini et al., 2018; Tomassini, Johansen-berg, et al., 2012). Notably, the expression levels of some neurotrophic factors, such as the nerve growth factor and the brain-derived neurotrophic factor, the increase of spine density and the recovery of the physiological synaptic function, were reported to be the fundamental parameters that influence the effects of corticospinal plasticity during the exercise (Ksiazek-Winiarek et al., 2015; Prosperini & Filippo, 2019).

Exercise increases clinical condition of pwMS, including balance, gait, muscle strength, cognitive functions, as well as it is a fundamental factor to increase corticospinal plasticity. To our knowledge, corticospinal plasticity is exercise-dependent, which is influenced by various parameters of exercises (Zentgraf & Helm, 2020). Aerobic exercise, promotes plastic changes due to modulation of angiogenesis and activation of glial cells, and due to the production of the nerve growth factor and the brain-derived neurotrophic factor (Devasahayam et al., 2017; Diechmann et al., 2021; El-Sayes et al., 2019; Stellmann et al., 2020). Resistance training, increases corticospinal plasticity via enrichment of the corticospinal output to both trained and untrained body limbs (Learmonth & Motl, 2021; Pickersgill et al., 2022).

Additionally, interlimb coordination of the upper limbs has been identified as an important factor in increasing the corticospinal plasticity (Cauraugh & Summers, 2005; Garry et al., 2005; Sun & Zehr, 2019). Studies involved both healthy individuals and

chronic stroke survivors have assessed corticospinal plasticity using TMS and concluded that interlimb coordination, particularly in-phase bilateral movement, has the strongest influence on corticospinal plasticity (Cauraugh & Summers, 2005; Whitall et al., 2011). The effect of in-phase bilateral movement on corticospinal plasticity can be attributed to the suppression of intracortical inhibition (Liepert et al., 2005; Stinear & Byblow, 2002) and the synchronized activation of homologous motor representations in M1. This process is facilitated by interhemispheric communication through transcallosal connections between M1 and Supplementary Motor Area (SMA) (McCombe Waller & Whithall, 2008; Toyokura M et al., 2002; Whitall et al., 2011).

1.13.2 Interlimb Coordination and Bilateral Movement

Bilateral movement refers to movements that require the simultaneous action of both limbs (Cuadrado M & Arias J, 2001; Toyokura M et al., 2002), with bilateral upper limb exercises shown to have positive neural effects on both hemispheres of the brain. Several TMS-based studies have identified three primary mechanisms explaining the efficacy of bilateral upper limb exercise (McCombe Waller & Whithall, 2008; Wu et al., 2021).

The first mechanism involves **transcallosal pathways**, which account for the effects of bilateral upper limb exercises on corticospinal plasticity. This type of exercise reduces intracortical inhibition, as evidenced by a decrease in MEP amplitude and enhances intracortical facilitation, as indicated by an increase in MEP amplitude, in both hemispheres (Meintzschel et al., 2002). In contrast, unilateral upper limb exercises produce the opposite phenomenon (McCombe Waller & Whithall, 2008). Intracortical inhibition refers to the ability of the M1 on one side of the brain to suppress mirror movements in the opposite M1 via transcallosal pathways, thereby reducing unintended motor output. Conversely, intracortical facilitation enhances motor output through these same pathways (Meintzschel et al., 2002).

In addition to the transcallosal pathways, two other mechanisms support the efficacy of bilateral upper limb exercises in stroke rehabilitation. Firstly, the **cortical projections to bilateral brainstem pathways** (e.g., rubrospinal or propriospinal pathways) and secondly, the **ipsilateral uncrossed corticospinal pathways.** Activating these pathways provides an adaptive strategy for motor recovery, enhancing motor function through the activation of the non-lesioned hemisphere (McCombe Waller & Whithall, 2008).

Bilateral movements play a crucial role in ADL and motor coordination, engaging both hemispheres of the brain to facilitate smooth and efficient movement. These movements can be categorized into three distinct types: in-phase, anti-phase, and complementary bilateral movements (Figure 1.15). Each type involves unique neuromuscular coordination and brain activation patterns, influencing how we perform everyday tasks. Understanding these movement patterns provides valuable insights into their functional significance and potential applications in motor recovery and rehabilitation.

In-phase bilateral movement involves both limbs moving in the same direction at the same time, activating the same muscle groups and corresponding brain regions in both hemispheres. In-phase bilateral movements are common in ADL, such as carrying and lifting large objects, pushing a cart, folding a towel, or catching thrown objects (Figure 1.15A). **Anti-phase bilateral** movement requires both limbs to move simultaneously in opposite directions, activating opposing muscle groups (agonists and antagonists) while engaging the same brain regions in both hemispheres (Figure 1.15B). Examples include swinging the arms while walking, climbing stairs, or driving (Smith & Richard Staines, 2010). **Complementary bilateral** movement, involves cooperative actions of both limbs to complete a task, with each limb performing a distinct function. For example, one hand may stabilize an object while the other manipulates it, such as when opening a bottle of water (McCombe Waller & Whithall, 2008). These findings highlight the neural mechanisms and functional applications of bilateral upper limb exercises, emphasizing their potential in motor recovery and rehabilitation.

Figure 1.15. In-phase vs antiphase bilateral hand movement.

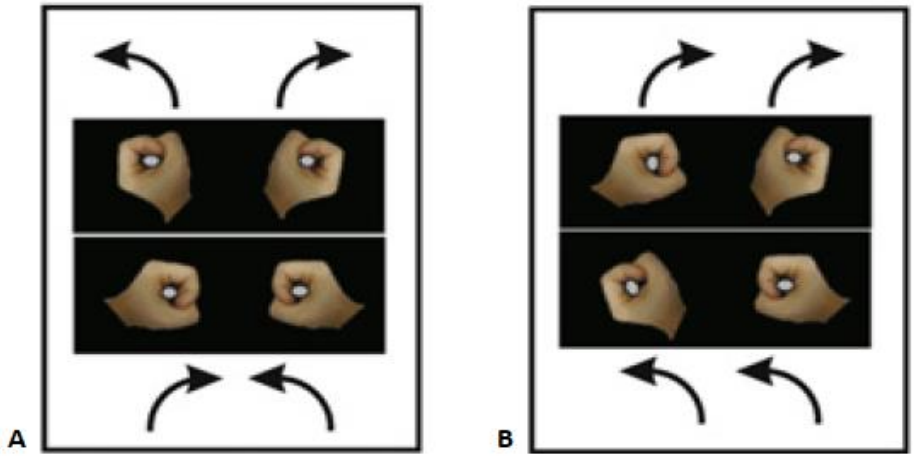


Figure 1.15: A. In-phase bilateral movement with flexion of both hands. B. Anti-phase bilateral movement with flexion of the right hand and extension of the left hand (Neva et al., 2012).

1.14 In-Phase Bilateral Movement: Neural Mechanism and Effects on Corticospinal Plasticity

To our knowledge, unilateral activation of upper limbs produces inhibition of the ipsilateral limb and prevention of mirror movements of the opposite upper limb, controlled throughout the interhemispheric inhibition (Duque et al., 2005). In contrast, during in-phase bilateral movements, both hemispheres are activated simultaneously, resulting in a reduction of intracortical inhibition (Figure 1.16) (Stinear & Byblow, 2002).

Figure 1.16. Intracortical interaction during in-phase bilateral movement.

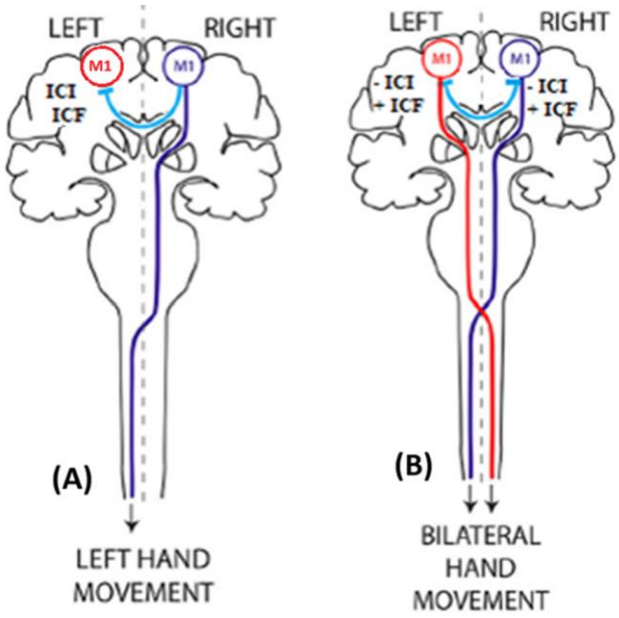


Figure 1.16: M1; primary motor cortex, ICI; intracortical inhibition ICF; intracortical facilitation. A. During unilateral hand movement there is a reduction of ICI and increase of ICF of the ipsilateral brain hemisphere. B. During in-phase bilateral movement both brain hemispheres are activated, causing reduction of ICI and increase of ICF.

Previous TMS-based studies on healthy individuals and chronic stroke survivors have demonstrated that in-phase bilateral movements disinhibit homologous muscle representations within M1, whereas anti-phase bilateral movements increase inhibition within the same M1 (Stinear & Byblow, 2002, 2004). In-phase bilateral movement induces corticospinal plasticity throughout activation of the same neural pathways in both brain hemispheres, because of the simultaneous activation of the homologous muscle groups (Cauraugh & Summers, 2005; Cohen, 1970; Hallett, 2007; Lacroix et al., 2004;

Smith & Richard Staines, 2010) and due to great cortical disinhibition (Liepert et al., 2000).

Several studies exploring the effects of in-phase bilateral movements have reported strong temporal and spatial interactions between the upper limbs. These include amplitude coupling (a tendency for both limbs to adopt similar movement amplitudes, even when assigned different amplitudes) and directional coupling (both limbs moving in the same direction), which further enhance corticospinal plasticity effects (Liepert et al., 2005).

Evidence from Wenderoth et al. (2004), suggested that the left brain hemisphere plays a dominant role in organizing mirror-symmetrical movements, while the right hemisphere specializes in coordinating non-mirror movements. This differentiation is mediated by intracortical inhibition through the corpus callosum (Wenderoth et al., 2004).

During in-phase bilateral movements, a central mechanism regulates the coordination of both upper limbs as a unified unit, facilitated by the bilateral projections of the SMA to both ipsilateral and contralateral neural networks. This mechanism is driven by interhemispheric crosstalk, which operates at two levels (Figure 1.17) (Cauraugh & Summers, 2005).

1. High-level crosstalk involves the transmission of movement parameters between the two hemispheres via the corpus callosum. The anterior portion of the corpus callosum connects the SMA and premotor cortices, while the posterior portion connects the parietal cortices. Callosal connections play a crucial role in the implementation of in-phase bilateral movement, because they provide a solid interhemispheric connection. The anterior portion of the corpus callosum connects the SMA and the premotor cortices, whereas the posterior portion connects the parietal cortices (Marconi et al., 2003).

2. Low-level crosstalk refers to the execution of in-phase bilateral movements via descending signals from the corticospinal tract. Although most corticospinal axons cross to the contralateral side at the medulla, approximately 10% remain uncrossed and project to distal limb muscles through the anterior corticospinal tract (Kuypers, 1982)

Figure 1.17. The interhemispheric crosstalk.

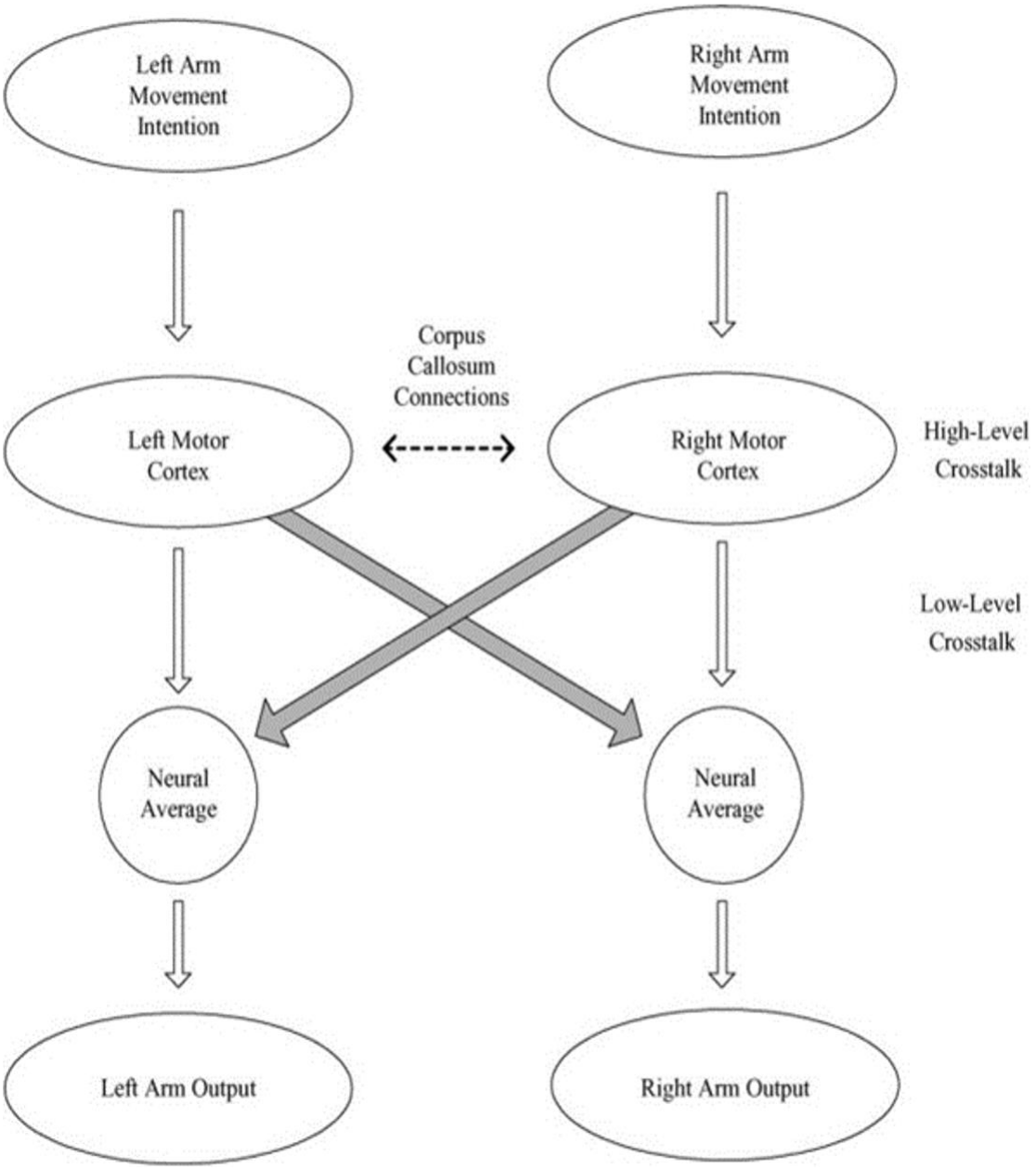


Figure 1.17: Two levels of the interhemispheric crosstalk presented in order to implement in-phase bilateral movement (i.e. high- and low-level crosstalk). Picture retried form (Cauraugh & Summers, 2005).

To our knowledge, in patients with neurological disorders, particularly chronic stroke survivors, there is evidence of an upregulation in the corticospinal tract. This occurs because, during the recovery process, the descending signals from the impaired upper limb often exceed those from the intact upper limb (Cauraugh & Summers, 2005). To address this imbalance, many researchers have sought to identify mechanisms that could

effectively improve motor function in this clinical population, particularly by leveraging the impact of corticospinal plasticity (Moucha R & Michael P, 2006).

Although, corticospinal plasticity is influenced by multiple factors, including various types of exercises, such as aerobic and resistance training (Diechmann et al., 2021; Learmonth & Motl, 2021; Sandroff et al., 2020) several studies in chronic stroke survivors indicated that in-phase bilateral exercises have the most pronounced effect on corticospinal plasticity (Garry et al., 2005; Neva et al., 2012; Stinear & Byblow, 2002; Sun & Zehr, 2019; Whitall et al., 2011). This effect is attributed to the suppression of intracortical inhibition and the simultaneous activation of homologous brain regions in both M1 areas (Cauraugh & Summers, 2005; Stewart et al., 2006).

In healthy individuals, TMS-based studies have shown that bimanual hand coordination is associated with symmetrical facilitation of neural activity, mediated by increased interhemispheric connectivity and enhanced transcallosal coupling between the SMA and M1 (Asemi et al., 2015; Grefkes et al., 2008; Rudisch et al., 2020; Swinnen & Wenderoth, 2004). Grefkes et al. (2008), specifically highlighted the efficacy of in-phase bilateral upper limb movements in enhancing interhemispheric connectivity between SMA and M1 in both hemispheres (Figure 1.18) (Grefkes et al., 2008).

Figure 1.18. Interhemispheric connectivity between supplementary motor area and primary motor cortex.

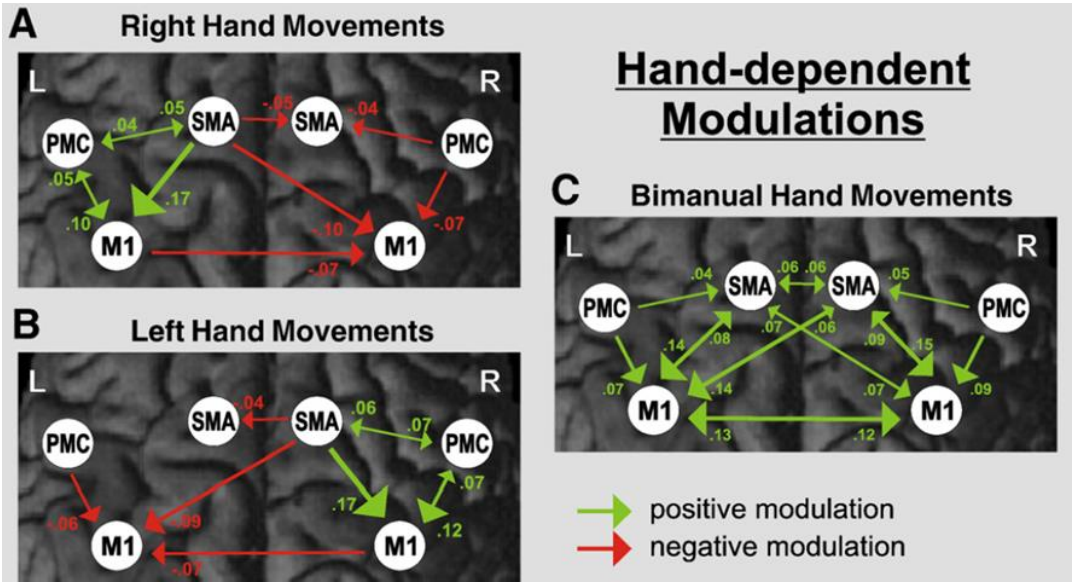


Figure 1.18: L; Left brain hemisphere, R; Right brain hemisphere. PMC; Pre-motor cortex, SMA; Supplementary motor area, M1; Primary motor cortex. Modulatory effects of upper limbs

movements on effective connectivity when moving the right (A), left (B) hand or both hands (C). Arrows (green: facilitation, red: inhibition) indicate modulated pathways for right, left and bilateral hand movements. Unilateral upper limb movements encourage mechanisms which suppress motor activation of the resting hand, indicating ipsilateral to the moving hand neural activity of M1. On the other hand, in-phase bilateral movements promote interhemispheric effective connectivity between SMA and M1 for both hemispheres (Grefkes et al., 2008).

During in-phase bilateral hand movements, where both M1 areas are synchronously activated, there is a positive coupling between M1 and SMA, resulting in increased connectivity within the motor network. This concurrent bilateral activation leads to positive neural modulation and enhances interhemispheric facilitation, contributing to improved motor function.

Throughout the literature, bilateral movements have a close relationship with cognitive functions in healthy individuals and pwMS (Grefkes et al., 2008; Rudisch et al., 2020; Swinnen & Wenderoth, 2004; Vasylenko et al., 2018b). The studies of Vasylenko et al. (2018), showed that bimanual dexterity in healthy older adults has a significant relationship between manual dexterity, age, gender and cognitive abilities. Specifically, bimanual dexterity performance declines with age (i.e., reduced performance in elderly people), shows gender differences (females tend to outperform males) and correlates with cognitive functions (Vasylenko et al., 2018a, 2018b).

Impairments in information processing speed are directly associated with other cognitive domains, such as working memory and attention (Genova et al., 2012; Leavitt et al., 2011; Lengenfelder et al., 2006), as well as with various motor skills, including eye-hand coordination and manual dexterity. Evidence from previous studies in both healthy individuals and pwMS indicated a strong relationship between cognitive functions and upper limb performance (Einarsson et al., 2006; Kierkegaard et al., 2012; Raats et al., 2018; Yozbatiran et al., 2006). This relationship is supported by dense neural projections from the anterior cingulate cortex (Figure 1.19) to the motor cortex and spinal cord (Asemi et al., 2015; Bush et al., 2000; Paus, 2001).

Figure 1.19. Anterior cingulate cortex anatomy.

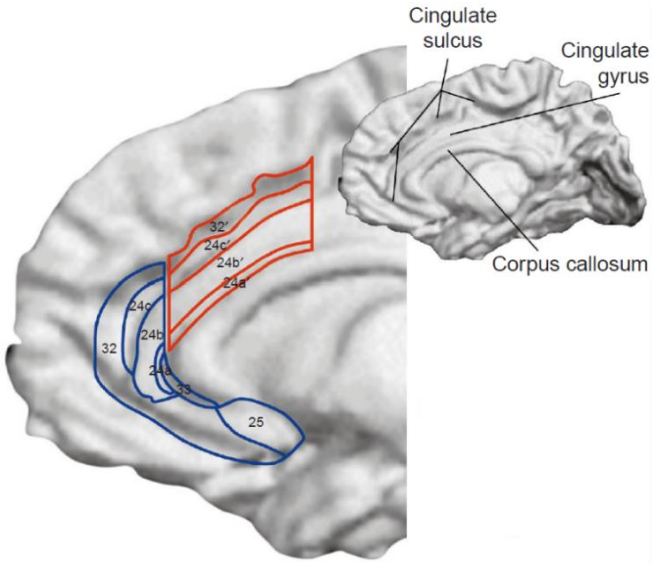


Figure 1.19: A schematic representation of cytoarchitectural areas (numbered) of anterior cingulate cortex anatomy (left schema). Areas 24b′, 24c′, 32 represent the dorsal cognitive division of the anterior cingulate cortex. The upper right part of the figure contains a reconstructed MRI of the medial surface of the right hemisphere of a single human brain (anterior towards the left, posterior towards the right). The cortical surface has been "partially inflated" to allow simultaneous viewing of gyri and sulci. Picture retrieved from (Bush et al., 2000).

Specifically, a decline in information processing speed performance is associated with reduced manual dexterity in pwMS (Poirier, 1988). Manual dexterity refers to the ability to perform coordinated fine and gross voluntary movements with the upper limbs (Poirier, 2015). Dysfunction in manual dexterity among individuals with MS significantly impacts their ability to perform ADL and engage in social activities, ultimately reducing independence and QoL (Yozbatiran et al., 2006).

Moreover, it is well established that exercise enhances cognitive functions in pwMS through mechanisms such as neuroprotection, neuroplasticity and neurodegeneration (White & Castellano, 2008). Specifically, previous cross-sectional studies—both objective and self-reported—have shown that physical activity can improve information processing speed (Li et al., 2023; Morrison & Mayer, 2017; Motl et al., 2011; Sandroff, Balto, et al., 2016; Vanner et al., 2008).

Finaly, several studies have demonstrated that greater muscle strength, balance and cardiorespiratory fitness are associated with improved information processing speed performance (Motl et al., 2013; Sandroff, Balto, et al., 2016; Sandroff & Motl, 2012).

These findings emphasize the importance of physical fitness in enhancing cognitive and motor functions in pwMS.

1.15 Rationale for the Research Program

1.15.1 Knowledge Gaps and Need for the Research Studies

It is well known that pwMS are classified as either having RRMS or PMS. However, pwRRMS frequently transition to PMS over time, leading to a substantial decline in their QoL. The literature suggests that optimizing corticospinal plasticity is a key factor in the management of RRMS. In contrast, pwPMS, who often experience pronounced cognitive dysfunction, may benefit from cognitive-motor strategies aimed at enhancing QoL.

Previous research in both healthy individuals and chronic stroke survivors has demonstrated significant improvements in corticospinal plasticity through simultaneous activation of the left and right M1. Clinical studies have validated these findings using in-phase bilateral upper limb exercises. Additionally, various cognitive rehabilitation interventions have been shown to effectively mitigate MS-related cognitive impairments and improve QoL. Notably, in-phase bilateral exercises have been identified as requiring the lowest cognitive demand among interlimb coordination exercises, making them potentially advantageous for individuals with cognitive deficits.

Despite extensive research on the effects of exercise on corticospinal plasticity in pwRRMS, the potential of in-phase bilateral exercises to induce motor-related neuroplastic adaptations in this population remains unclear. Furthermore, for pwPMS an important question arises: Could specific exercise modalities serve as an alternative to conventional cognitive rehabilitation, simultaneously enhancing specific cognitive domains and motor function?

Given that MS is often characterized by bilateral hemispheric lesions leading to both motor and cognitive impairments, and considering the established efficacy of in-phase bilateral upper limb exercises in promoting corticospinal plasticity, these exercises may offer therapeutic benefits for pwMS. In RRMS, such interventions may contribute to neuroplasticity preservation across both hemispheres, which is crucial for disease management during both relapse and remission phases. Moreover, physical exercise is often more engaging and widely accessible than conventional cognitive training. For

individuals with PMS, who frequently experience deficits in cognitive processing, incorporating low-cognitive-demand exercises may provide dual benefits—enhancing cognitive function while concurrently improving motor performance.

1.15.2 Link Between Corticospinal Plasticity, Cognitive Function, and Bilateral Movement

Corticospinal plasticity plays a crucial role in motor function recovery and adaptation in MS. It refers to the brain's ability to reorganize corticospinal pathways in response to external stimuli, such as physical activity or injury. Individuals with RRMS, fluctuations in corticospinal excitability are closely associated with clinical recovery post-relapse, making corticospinal plasticity a key biomarker of motor function improvement. Similarly, in PMS, cognitive impairments—particularly deficits in information processing speed—are a predominant challenge, significantly affecting QoL. Notably, both corticospinal plasticity and cognitive function are exercise-dependent, suggesting a shared neurophysiological basis that can be targeted through specific motor training interventions.

Bilateral movement, particularly in-phase bilateral upper limb exercises, has been extensively studied for its role in enhancing both corticospinal plasticity and cognitive processing. These exercises involve simultaneous activation of both M1, facilitating interhemispheric communication and enhancing corticospinal excitability (McCombe Waller & Whitall, 2008). Research in chronic stroke survivors and healthy populations has demonstrated that interlimb coordination exercises can promote neuroplasticity, likely through mechanisms such as interhemispheric facilitation and recruitment of shared motor and cognitive networks (Garry et al., 2005; Sun & Zehr, 2019). Given that corticospinal plasticity in RRMS and information processing speed in PMS are both modifiable through exercise (Gharakhanlou et al., 2021; Neva et al., 2022; Sandroff, Motl, et al., 2016; Zeller & Classen, 2014), bilateral movements may serve as a therapeutic approach for improving both motor and cognitive functions in pwMS.

Furthermore, in-phase bilateral exercises are associated with lower cognitive demand compared to other interlimb coordination tasks, making them particularly suitable for pwPMS, who frequently experience cognitive processing deficits. The simultaneous activation of both M1 regions not only strengthens corticospinal pathways but may also

engage higher-order cognitive networks involved in attention and executive function, thereby improving information processing speed. This dual benefit of enhancing both motor and cognitive outcomes highlights the potential of bilateral movement-based interventions as an accessible and effective rehabilitation strategy for pwMS.

1.16 Research Aim, Questions, Hypotheses and Thesis Progression

1.16.1 Overall Aim

The overarching aim of this thesis is to investigate the effects of in-phase bilateral upper limb exercises on corticospinal plasticity and clinical outcomes in pwMS. Specifically, this research seeks to examine whether the specific type of exercises, which simultaneously activate both M1, can enhance corticospinal plasticity in pwRRMS and improve information processing speed in pwPMS. Given that corticospinal plasticity is a key biomarker of motor recovery in RRMS and that cognitive dysfunction, particularly deficits in information processing speed, is a primary challenge in PMS, this thesis aims to explore the potential of in-phase bilateral upper limb exercises as a dual-modality intervention targeting both motor and cognitive impairments.

By leveraging the neurophysiological principles of interhemispheric facilitation and exercise-induced plasticity, this research will assess whether in-phase bilateral exercises can serve as a complementary or alternative rehabilitation strategy to conventional motor and cognitive training in pwMS. Ultimately, the findings of this thesis will contribute to a better understanding of the mechanisms underlying exercise-induced neuroplasticity and inform the development of targeted rehabilitation strategies aimed at improving QoL in this clinical cohort.

1.16.2 Specific Research Questions and Hypotheses for Each Study

This thesis aims to investigate the effects of in-phase bilateral upper limb exercises on corticospinal plasticity and clinical outcomes in pwMS. Specifically, it examines whether this type of exercise can enhance corticospinal plasticity in pwRRMS and improve information processing speed in pwPMS. The study is guided by the following research questions and hypotheses:

The first research question is whether in-phase bilateral upper limb exercises can enhance corticospinal plasticity in pwRRMS, along with contributing to improvements in clinical outcomes. In line with this question, it is hypothesized that in-phase bilateral upper limb exercises will lead to a significant increase in corticospinal plasticity in pwRRMS, as assessed by TMS and will result in significant improvements in individual clinical symptoms as measured by different clinical assessment tools.

The second research question is whether in-phase bilateral upper limb exercises can improve information processing speed in pwPMS and whether any potential improvements are correlated with manual dexterity. Based on this question, it is hypothesized that in-phase bilateral upper limb exercises will provide a dual benefit by improving both motor and cognitive function in pwPMS. Specifically, improvements in information processing speed are expected, and, given the strong relationship between information processing speed and upper limb performance, an increase in manual dexterity functioning is also anticipated to correlate with improvements in information processing speed within this type of MS.

By addressing these research questions and testing these hypotheses, this thesis aims to provide novel insights into the role of in-phase bilateral exercises in enhancing corticospinal plasticity and functional outcomes in pwMS. The findings from this research may contribute to the development of targeted rehabilitation strategies tailored to the specific needs of individuals with different MS subtypes.

1.16.3 Thesis Progression

Building on the previous mentioned evidences, the present thesis aims to investigate the impact of in-phase bilateral upper limb exercises on corticospinal plasticity and clinical outcomes in pwRRMS, as well as their potential to enhance information processing speed in pwPMS. By examining the interplay between motor and cognitive function in response to bilateral movement training, this research seeks to provide novel insights into exercise-based neurorehabilitation strategies tailored to the specific needs of pwMS.

To address the research aims, this thesis follows a structured progression that includes three distinct phases of investigation, each designed to build upon the findings of the preceding stage. The research employs both single-case and group study designs to assess

the effects of in-phase bilateral upper limb exercises on neuroplasticity and clinical outcomes in pwMS.

Pilot Single-Case Study

The first phase of the research involved a pilot single-case study conducted with one patient diagnosed with RRMS (n=1). This study aimed to assess the potential effects of an in-phase bilateral upper limb exercise protocol on corticospinal plasticity and the clinical condition of the patient. Several neurophysiological TMS-based measures and clinical assessments were performed to evaluate changes in corticospinal plasticity and clinical symptoms. The pilot study was conducted to identify potential methodological limitations in both neurophysiological and clinical assessments, as well as any possible adverse effects or difficulties during the implementation of the specific exercise protocol. Also, the results of this pilot study confirmed the feasibility of this methodology, which provided the foundation for the subsequent research in the thesis (Chapter 2.2).

Concurrent Multiple Baseline Design Study

Following the pilot study, the research progressed to the first clinical trial, utilizing a concurrent multiple baseline design across five pwRRMS (n=5). In this phase, the same exercise protocol used in the pilot study was applied. The primary objective was to observe changes in corticospinal plasticity and clinical condition in pwRRMS. Neurophysiological TMS-based measures and clinical assessments were employed to determine whether in-phase bilateral upper limb exercises could enhance corticospinal plasticity and improve both cognitive and motor outcomes. This phase addressed the first research question and provided valuable insights into the potential of the intervention to improve neuroplasticity and clinical outcomes in pwRRMS (Chapter 2.3).

Group Comparison Study

Given that most pwRRMS eventually transition to the progressive type of MS, and considering the substantial number of individuals already affected by this form, a second clinical trial was conducted to address the second research question. A group comparison study was conducted with twenty individuals diagnosed with PMS (n=20). The participants were allocated (1:1) to either the experimental group (n=10) or the active control group (n=10). The experimental group performed the in-phase bilateral upper limb exercise program, while the control group followed a conventional exercise routine.

he primary objective of this study was to investigate the effects of in-phase bilateral upper limb exercises on information processing speed and its potential correlation with manual dexterity performance, along with examining other clinical outcomes. The results of this group comparison study provided further insights into the potential dual benefits of the intervention for both cognitive and motor functions in pwPMS (Chapter 3).

By systematically progressing through these stages, this thesis contributes to the understanding of how in-phase bilateral upper limb exercises can influence corticospinal plasticity and improve both motor and cognitive outcomes in individuals with different forms of MS. The findings from each phase build on one another, offering novel insights into the potential of exercise-based interventions for pwMS.

1.17 Overview of Thesis' Chapters

1.17.1 Chapter 2: Studies on the Effects of In-Phase Bilateral Exercises in Relapsing-Remitting Multiple Sclerosis (RRMS)

RRMS is the most prevalent form of MS, characterized by episodic relapses followed by periods of partial or complete remission. Acute motor relapses in RRMS are associated with bi-hemispheric alterations in corticospinal excitability and inflammatory lesions, contributing mainly to motor and cognitive impairments that affect the QoL of pwRRMS.

Motor dysfunction in RRMS is closely linked to changes in corticospinal tract integrity and neuroplasticity. The corticospinal tract, a principal motor pathway responsible for voluntary motor control, undergoes neuroplastic changes that influence post-relapse clinical recovery. Several TMS-based neurophysiological studies assess corticospinal plasticity through excitability measures such as rMT, MEP amplitude and latency, CMCT. These measures provide insight into both excitability and conduction efficiency of the corticospinal tract in RRMS.

Corticospinal plasticity is modulated by various exercise modalities, including aerobic and resistance training, as well as interlimb coordination exercises. Prior research indicates that in-phase bilateral movements significantly influence corticospinal plasticity through interhemispheric mechanisms. Given the presence of bilateral cortical lesions in RRMS, it remains unclear whether in-phase bilateral exercises can induce bilateral neuroplastic adaptations in this population. Therefore, the first clinical trial study was

conducted which aimed to evaluate the impact of an in-phase bilateral exercise protocol, adapted to sports activities and functional training, on corticospinal plasticity and clinical outcomes in pwRRMS.

This study consisted of two research components. First, a single-case pilot study was conducted with one participant with RRMS to identify methodological challenges and assess feasibility. This was followed by a registered clinical trial, which used a single-case concurrent multiple baseline design across five participants with RRMS. The design allowed for a staggered baseline phase, enabling the inference of causal relationships between the intervention and changes in corticospinal plasticity. Corticospinal excitability was evaluated bilaterally using TMS, while clinical assessments included measures of gait, balance, strength, hand dexterity, cognitive function, fatigue, and QoL.

1.17.2 Chapter 3: Study on the Effects of In-Phase Bilateral Exercise in Progressive Multiple Sclerosis (PMS)

PMS, encompassing Secondary Progressive MS and Primary Progressive MS, is a debilitating stage of MS marked by increasing neurological impairment and disability. Since most pwRRMS eventually transition to the progressive stage of the disease, identifying effective treatment options is crucial. Unfortunately, current therapies offer limited benefits at this stage, emphasizing the need for targeted interventions.

Cognitive impairment, particularly slowed information processing speed, is a major challenge for pwPMS, significantly affecting manual dexterity and QoL. Research suggests that physical exercise, especially in-phase bilateral upper limb movements, can enhance cognitive and motor function by strengthening interhemispheric communication.

Based on the literature of PMS and building on the prior clinical trial in pwRRMS, a second registered clinical trial was conducted to investigated whether a 12-week in-phase bilateral upper limb exercise program could improve information processing speed and other clinical measures in pwPMS.

1.17.3 Chapter 4: General Discussion and Conclusion

Overall, the findings from both clinical trial studies support the integration of in-phase bilateral exercises into neurorehabilitation programs for pwMS, offering a promising strategy to enhance cognitive and motor functions, as well as corticospinal plasticity.

While the in-phase bilateral upper limb exercise protocol did not alter the CMCT in RRMS, it led to a bilateral reduction in rMT, indicating enhanced corticospinal plasticity. Despite unchanged CMCT, participants experienced improvements in motor and cognitive functions, supporting the clinical relevance of this intervention. In pwPMS, the in-phase bilateral upper limb exercise protocol significantly improved information processing speed, manual dexterity and clinical outcomes. Enhanced performance on the Symbol Digit Modalities Test and the Purdue Pegboard Test suggests improved cognitive-motor integration through interhemispheric communication. Additionally, despite focusing on the upper limbs, the specific exercise protocol improved gait, balance, fatigue and QoL in pwPMS, highlighting its holistic benefits.

The current thesis acknowledges several methodological limitations, including small sample sizes, the lack of neuronavigation in TMS assessments, the absence of follow-up in the second clinical trial, and the varying exercise frequencies between groups. Future research should investigate long-term effects, optimize training protocols, and incorporate neuroimaging to confirm the underlying mechanisms.

1.18 Chapter Summary

MS is a chronic autoimmune neurodegenerative disease characterized by demyelination and neurodegeneration. MS can manifest as RRMS, the most common form, or PMS. A wide range of clinical symptoms are exhibited in pwMS, primarily including motor and cognitive dysfunctions. Motor impairments are closely connected to corticospinal plasticity, which can be assessed using TMS, whereas cognitive deficits, particularly information processing speed, are prominent in pwPMS. It is well-established that corticospinal plasticity, motor and cognitive functions are influenced by exercise. Furthermore, interlimb coordination has been shown to have a significant relationship with neuroplastic changes and various cognitive functions. Evidence from previous research on healthy individuals and chronic stroke survivors has demonstrated the effectiveness of simultaneous bilateral activation of M1 through in-phase bilateral upper limb movements in improving both corticospinal plasticity and information processing speed. Therefore, the aim of this thesis is to explore the effects of in-phase bilateral upper limb exercises in both RRMS and PMS. The following chapters outline the steps taken to address the research questions of this thesis.

CHAPTER 2:

Studies on the Effects of In-Phase Bilateral Exercises in Relapsing-Remitting Multiple Sclerosis

2.1 Introduction

Relapsing-Remitting Multiple Sclerosis (RRMS) is the most common type of Multiple Sclerosis (MS) and is characterized by periods of relapses followed by partial or complete recovery (Lublin et al., 2014). After an acute motor relapse, bi-hemispheric changes of corticospinal excitability and inflammatory lesions could be observed (Chieffo et al., 2019; Kister et al., 2013; Lublin et al., 2020) resulting in diverse clinical condition and symptoms. Individual symptoms in people with RRMS (pwRRMS) include motor and cognitive impairments, visual deficits, depression and fatigue (Benedict et al., 2020; Kister et al., 2013; Norbye et al., 2020). These symptoms result in significantly low quality of life (QoL) (Frndak et al., 2015; Strober et al., 2014) which subsequently cause the need for lifelong support and management of symptoms for most pwRRMS (Fortune et al., 2021).

Motor symptoms in RRMS are associated with changes in corticospinal tract integrity and neuroplasticity (Flachenecker, 2015; Fritz et al., 2017; Lipp & Tomassini, 2015; Pawlitzki et al., 2017; Tavazzi et al., 2021; Tomassini, Matthews, et al., 2012). The corticospinal tract is one of the major motor descending pathways providing voluntary motor function in humans (Lemon, 2008). The neuroplasticity of the corticospinal tract, is defined by changes in neuron structure or function, detected either directly from measures of individual neurons or inferred from measures taken across populations of neurons (Warraich & Kleim, 2010) and is an essential factor that predicts clinical recovery in the post-relapse stage of pwRRMS (Mori et al., 2013, 2014). Corticospinal plasticity can be probed using Transcranial Magnetci Stimulaiton (TMS) (Neva et al., 2016; Pascual-Leone A et al., 1998; Pascual-Leone et al., 1995) and characterized via corticospinal excitability measures including resting motor threshold (rMT), motor evoked potentials (MEPs) amplitude and latency, and the central motor conduction time (CMCT) (Snow et al., 2024). Motor threshold and MEPs amplitude are the hallmark measures of corticospinal excitability in MS (Bestmann & Krakauer, 2015), whereas the MEPs latency and CMCT are temporal measures of the corticospinal excitability (Chen et al., 2008).

Corticospinal plasticity is exercise-dependent (Neva et al., 2022; Zeller & Classen, 2014) and influenced by various factors (Cardoso et al., 2024; Moucha R & Michael P, 2006;

Prosperini & Filippo, 2019), such as aerobic exercise (DeLuca et al., 2020; Diechmann et al., 2021; Learmonth & Motl, 2021), resistance training (Learmonth & Motl, 2021; Tavazzi et al., 2021), as well as interlimb coordination (Garry et al., 2005; Sun & Zehr, 2019). Previous studies that assessed corticospinal plasticity using TMS in healthy participants and in chronic stroke survivors, reported that interlimb coordination and especially in-phase bilateral movement has the strongest effect on corticospinal plasticity (Neva et al., 2012; Smith & Richard Staines, 2010; Stinear & Byblow, 2002; Whitall et al., 2011). These effects are thought to be due to the suppression of cortical inhibition (Liepert et al., 2005; Stinear & Byblow, 2002) and the simultaneous activation of homologous representations of the motor cortices, which involves interhemispheric facilitation via transcallosal connection between primary motor cortex (M1) and the Supplementary Motor Area (SMA) (Cuadrado M & Arias J, 2001; Toyokura M et al., 2002).

Despite the broad literature on the effects of different types of exercises on neuroplasticity in pwRRMS (Akbar et al., 2020; DeLuca et al., 2020; Proschinger et al., 2022; Reina-Gutiérrez et al., 2022) it is unclear whether in-phase bilateral exercises can promote motor related neuroplastic changes in RRMS. In light of evidence that pwRRMS have bilateral cortical lesions (Calabrese et al., 2010) which cause bilateral changes of corticospinal tract integrity (Bergsland et al., 2015; Pawlitzki et al., 2017), these findings raise the question about the effects of in-phase bilateral exercises on corticospinal plasticity. Such effects would provide strong evidence about whether exercise, in particular in-phase bilateral exercise, can influence the corticospinal plasticity in RRMS.

The aim of this study was therefore to investigate whether the intervention protocol of in-phase bilateral exercises for the upper limbs, which were adapted to sports activities and to functional training (see page 80), could significantly affect the corticospinal plasticity and subsequently the individual clinical condition of pwRRMS. The primary hypothesis was that a significant improvement of corticospinal plasticity would detect bilaterally as measured with CMCT, caused by the specific intervention protocol which included in-phase bilateral exercises of the upper limbs, in pwRRMS. Corticospinal plasticity was assessed bilaterally using TMS and calculated corticospinal excitability measures (Neva et al., 2012). Additionally, further analyses examined the effects of the specific exercise protocol on rMT, MEP amplitude and latency, as well as on clinical symptoms using

various clinical assessments, including gait, balance, strength, hand dexterity, cognitive function, fatigue and QoL (Fisk, Ritvo, et al., 1994).

For the purpose of the current project, two research studies were performed. The first study, *a single case pilot study*, focused on recruiting one participant based on predefined inclusion and exclusion criteria. This pilot study was designed primarily to identify potential methodological issues, assess the feasibility of the research design and examine any limitations that might arise during the study process. A single-case approach was chosen for its ability to offer a detailed, in-depth examination of the individual participant's response to the intervention, allowing researchers to gain valuable insights into the intervention's effectiveness in a real-world setting. Moreover, the single case design provides a flexible and efficient method for identifying variables that might impact the study's outcome, such as participant characteristics or environmental factors, while also highlighting any issues that may need to be addressed in the second study, which utilized a single-case concurrent multiple baseline design involving five participants (Kratochwill et al., 2010).

The second study was a *single-case concurrent multiple baseline design* (Kratochwill et al., 2010) with five pwRRMS. This specific methodological design has the advantage to verify the cause-effect inference clearly by the staggered duration through separate baseline phases (Zhan & Ottenbacher, 2001). Consequently, possible effects from this study were expected to provide preliminary evidence and proof-of-concept evidence for this type of exercise, which can be applied during the disease progression and to existing neurorehabilitation protocols, in this particular clinical cohort.

Visual analysis was conducted separately for each variable and results are presented graphically according to the level, trend and stability, to define functional relationships between the intervention protocol and the corticospinal plasticity. Subsequently, a statistical analysis was performed only for the single-case concurrent multiple baseline design across five subjects, and for the single case pilot study, in all outcome measures, which indicated a sizeable effect from the visual analysis, to estimate the effect of the intervention and then randomization tests were constructed to evaluate statistical significance (Lobo et al., 2017).

2.1.1 Participants

All participants were patients registered in the outpatient registry at The Cyprus Institute of Neurology and Genetics. Individual medical records were collected prior to the beginning of the study and none of the researchers had access to them ensuring that individual participants could not be identified during or after data collection at The Cyprus Institute of Neurology and Genetics. Each participant had a unique patient file containing detailed examinations and clinical descriptions, as they regularly attended medical appointments and evaluations by neurologists and other healthcare providers at the institute. Therefore, all inclusion and exclusion criteria were confirmed by the neurologist who reviewed the individual medical records.

The inclusion criteria included 1) diagnosis of RRMS, 2) Expanded Disability Status Scale (EDSS) score between three and five (Kurtzke, 1983), 3) aged between 30 and 70 years, 4) no relapse within 30 days and 5) Mini Mental State Examination score between 24 and 30 (no cognitive impairment) (Folstein et al., 1975). The exclusion criteria included 1) brain metal implants (e.g., titanium skull plates, aneurysm clips) (Rossi et al., 2022), 2) history of any disease affecting the CNS other than MS (e.g., stroke, Parkinson's disease, cerebral palsy), 3) history of cardiovascular disease (e.g., known aneurism, myocardial infarction, hyper/hypotension, heart failure), 4) mental disorders (e.g., depression, schizophrenia, bipolar syndrome), 5) severe orthopaedic disorders (e.g., knee or hip replacement, spondylosurgery, disk herniation, recent bone fracture), 6) pregnancy during the implementation of the study timeline, 7) visual deficit (e.g., optic neuritis, blindness, diplopia, glaucoma, blurred vision), 8) hearing impairments (i.e., deafness), 9) history of epileptic seizures and 10) spasticity level on upper or lower limbs more than 1+ (slight increase in muscle tone) according to Modified Ashworth Scale (Meseguer-Henarejos et al., 2018).

Additionally, participants were advised to continue their usual prescribed medication throughout the study duration and they were advised to continue their usual routine and avoid receiving any other exercise program during the study. Furthermore, all participants read and signed a written informed consent, while all procedures were approved and conducted in accordance with the ethical guidelines of the Cyprus National Bioethics Committee before recruitment (ΕΕΒΚ/ΕΠ/2022/32) (Appendix II - Ethical Committee Approval).

2.1.2 Outcome Measures

Since prolongation of CMCT is the most common neurophysiological characteristic in pwMS and given the results of the study of Meng et al. (2018), which indicated short term improvement of the CMCT after bilateral exercises of the upper limbs in chronic stroke survivors (Meng G, Meng X, Tan Y, Yu J, Jin A, Zhao Y, 2018; Udupa & Chen, 2013), CMCT was designated as the primary outcome variable. CMCT expresses the time taken for neural impulses to reach from motor cortex to alpha-motoneurons (Udupa & Chen, 2013), which refer to the integrity of the white matter fibers (Stokes et al., 2020). Therefore, we calculated bilateral CMCT using both TMS and peripheral stimulation of the median nerve (see below; Data Acquisition of Outcome Measures) to observe possible changes in the central nervous system due to possible effects of the exercise protocol.

The secondary outcome measures included the rMT (states the general excitability of the neuromotor axis in the target muscle), the MEPs amplitude (expresses the trans-synaptic activation of corticospinal neurons) and latency (defines the time which is needed for signal transmission from the motor cortex to the recording electrode of the target muscle) (Rossini et al., 2015), and all clinical assessments. We quantified the rMT and the MEPs amplitude and latency using a single pulse TMS whereas several clinical assessments were performed to each participant.

2.1.3 Data Acquisition of Outcome Measures

We assessed the corticospinal plasticity using single-pulse TMS in the neurophysiology lab of The Cyprus Institute of Neurology and Genetics. Using EMG signals from an upper limb muscle (see below; EMG recording), we collected MEPs, which were used to calculate all corticospinal excitability measures. During all neurophysiological assessments, participants were in a relaxed sitting position in a comfortable chair with feet touching the floor and both arms placed on a cushion (Figure 2.1). To ensure methodological consistency, we collected all data by performing the same methodological procedures for both conditions (i.e., corticospinal excitability measures bilaterally) - one side per assessment- across participants and across all time points.

Figure 2.1. Transcranial Magnetic Stimulation assessment.

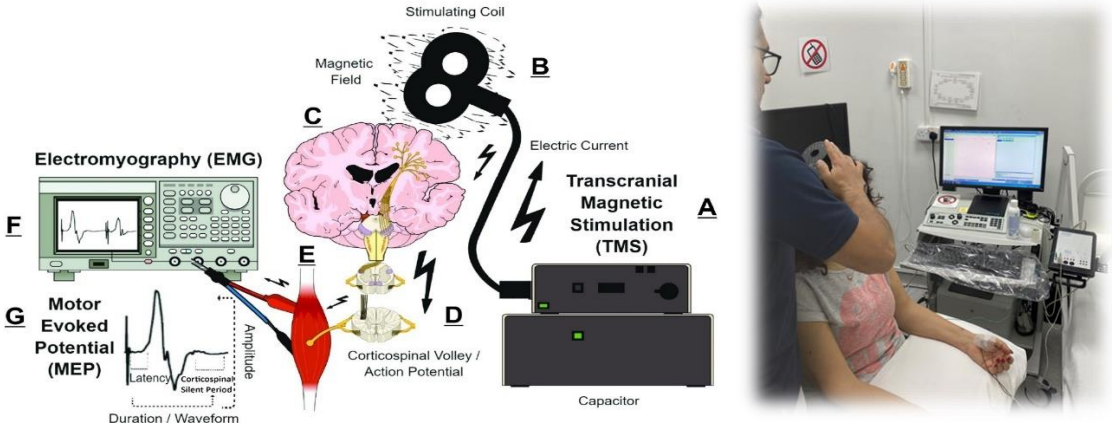


Figure 2.1: TMS; Transcranial Magnetic Stimulation, MEP; Motor evoked potential, EMG; Electromyography. **Left figure:** TMS schematic illustration of MEP generation. (A) Pulse generator produces an electric current, which it is discharged from the capacitor. (B) Figure-eight coil generates a focal magnetic field from the electric current. (C) The magnetic field undergoes from extracerebral tissues to the primary motor cortex. (D) Activation of descending corticospinal volleys from the brain to the spinal cord, by direct activation of the pyramidal tract neurons. (E) Activation of the target muscle via stimulation of motor unit and the peripheral nerve. (F) TMS-induces MEP can be recorded via EMG. (G) EMG waveform that contains the amplitude, latency, duration, and corticospinal silent period. **Right figure:** A real time TMS assessment as it was performed in the neurophysiology lab for the need of this thesis. A figure-eight coil was used to deliver all single-pulses, while the participant was in a relaxed sitting position in a comfortable chair with feet touching the floor and both arms placed on a cushion. The record electrodes were placed over the Abductor Pollicis Brevis.

EMG recording

During both TMS and peripheral stimulation, surface EMG of the Abductor Pollicis Brevis was collected. We followed a standard skin preparation (Hermens et al., 2000) and surface disk electrodes placement procedures by attaching the electrodes over the end plate region of the Abductor Pollicis Brevis (Hidasi et al., 2009). Specifically, the anode electrode was placed distally, whereas the cathode electrode proximally. A ground reference electrode was attached on the lateral condyle of the elbow, of the corresponding upper limb (Figure 2.2). Additionally, all signals were recorded with sampling rate of 24kHz and were filtered with a bandwidth of 2Hz–10 kHz using KeyPoint Net Software Electromyography (version 2.40; Natus Medical Incorporated G4, United States).

Figure 2.2. Electromyography electrodes placement on the Abductor Pollicis Brevis.

Figure 2.2: The electrodes were placed over the Abductor Pollicis Brevis. The anode (red colour) electrode was placed distally, whereas the cathode (black colour) electrode proximally.

Peripheral stimulation

In addition to MEPs latency, calculating the CMCT requires two peripheral derived measures, the *F*- wave (i.e., late muscle response) and the *M*- wave (i.e., direct muscle response) (Hallett, 2007; McNeil et al., 2013) Therefore, we initially delivered peripheral stimulation on the median nerve at the wrist, approximately in an 8 cm distance from the cathode electrode (Hidasi et al., 2009), while collecting EMG from the Abductor Pollicis Brevis (Figure 2.3) (Fisher, 2007).

Figure 2.3. Peripheral stimulation of the median nerve.

Figure 2.3: Delivery of peripheral stimulation on the median nerve at the left wrist.

Transcranial Magnetic Stimulation assessment

Following TMS recommended guidelines concerning safety and experimental conditions (Groppa et al., 2012; Rossini et al., 2015), we assessed bilateral corticospinal excitability measures. We applied TMS single pulses (Pascual-Leone et al., 1995) via figure-eight coil (C-B60; inner diameter: 35mm, outer diameter: 75mm), connected to the MagPro R20 (MagVenture User Guide, United Kingdom edition, MagVenture A/S, Denmark). The coil was oriented tangentially over the contralateral motor area of the brain, relative to the target muscle (i.e., Abductor Pollicis Brevis), with a posterolateral handle pointing in approximately 45 degrees angle to the sagittal plane inducing posterior-anterior current in the brain (Balloff et al., 2022).

For the TMS procedures, we first found the optimal stimulation site (i.e., hot-spot) without neuronavigation. To date, two main approaches for determining the hot-spot have been described in the literature. The first approach involves using a neuronavigation system, which allows precise identification of the hot-spot. These systems integrate imaging modalities, such as MRI, with real-time navigation to create a three-dimensional representation of the patient's brain (Figure 2.4). Conversely, an alternative method, known as the adaptive threshold-hunting method, can also be used to identify the hot-spot.

For the needs of this thesis, the adaptive threshold-hunting method was conducted. Firstly, it was ensured that each patient was sitting comfortable with the EMG electrodes attached to the Abductor Pollicis Brevis. Afterwards the vertex of the skull was based on brain's anatomical landmarks (i.e., nasion to inion, left to right tragus). Typically, the hand area of the M1, based on the Homunculus topographic representation, is located about 5cm lateral and 2cm anterior to the vertex. Therefore, to determine hot-spot (i.e., the spot in which the largest response of the target muscle is elicited), several single pulses were delivered within an imaginary isosceles triangle located over the hand area. Single pulse was firstly delivered at low intensities (e.g., ~20% MSO) and then gradually increased by 1% – 5% MSO until the intensity that elicited three consecutive MEPs with peak-to-peak amplitude greater than 50mV, was reached (Charalambous et al., 2018). Then, the position of the coil on the skull was marked with a water-resistant ink, to determine the rMT of the target muscle.

Figure 2.4. Localization of hot-spot without neuronavigation.

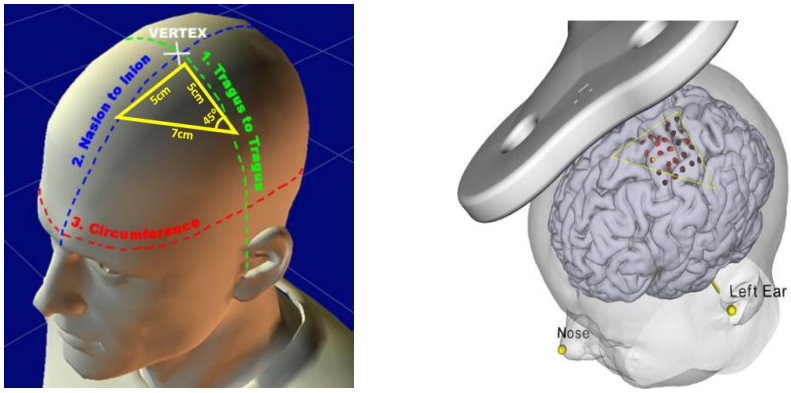


Figure 2.4: Illustration of the brain in the neuronavigation system. Despite the fact that we did not use neuronavigation system these two figures illustrate the procedure of the hot-spot localization over the hand area. **Left figure:** The vertex is located at the midpoint of the line between nasion and inion (blue dash line) and at the midpoint of the line between left and right tragus of the ears (green dash line). An imaginary isosceles triangle was created, with dimensions of 5cm from vertex to the ear and 5cm from vertex to the forehead, to localize the hand area (yellow colour) and to deliver several TMS single pulses. **Right figure:** Schematic illustration in neuronavigation, of how the several TMS single pulses can be seen within the imaginary triangle which is located over the hand area.

rMT is the minimum stimulation intensity needed to produce MEPs of the target muscle. To identify the rMT, we employed an adaptive threshold-hunting method, the Motor Threshold Assessment Tool (MTAT 2.0) (Awiszus, 2003) (available at http://clinicalresearcher.org/software.htm). The specific method has the advantage of speed without losing accuracy when compared to the relative-frequency methods based on the Rossini–Rothwell, although both methods have similar precision. Also, MTAT 2.0 allows for adaptive adjustments based on real-time feedback, ensuring that clinicians and researchers can find the motor threshold efficiently while minimizing unnecessary stimuli (Ah Sen et al., 2017; Dissanayaka et al., 2018). After the rMT was determined, a bout of single pulses was applied using suprathreshold stimulation to quantify the MEPs-derived measures of interest (i.e., MEPs amplitude and latency). Specifically, 30 suprathreshold stimuli were applied (Goldsworthy et al., 2016) at 120% of the rMT (Snow et al., 2019).

Clinical assessment

All clinical assessments were performed in the physiotherapy unit of The Cyprus Institute of Neurology and Genetics.

Mini Balance Evaluation Systems Test

It measures dynamic balance, functional mobility and gait in neurological patients, including pwRRMS (Franchignoni et al., 2010). The specific test consists of 14 items, including four of the six segments from the Balance Evaluation Systems Test (anticipatory postural adjustments, sensory orientation, reactive postural control and dynamic gait). The Mini Balance Evaluation Systems Test is scored out of 28 points to include 14 items that are scored from zero to two (Appendix III - Clinical assessments forms).

Six Spot Step Test

It is a timed walking test that involves kicking over a number of targets placed along a 5m-path (Figure 2.5) in which rely to some extent on vision and cognition (Nieuwenhuis et al., 2006). The Six Spot Step Test is measured in the time domain replicating a complex range of sensorimotor functions, part of which are lower limb strength, spasticity, coordination and balance. The 5m × 1m test field has plastic marking plates at each end and side-line (1m & 3m on one side, 2m & 4m on the other). Participants start with feet on a plate, walk between circles, and kick plates out with one foot as fast as possible without running. Timing starts when they lift their foot and ends after the last kick. Each participant completes four runs, two per leg, using only one foot per run (Nieuwenhuis et al., 2006). For the data analysis we recorded the mean time of the four runs as the final test result (Callesen et al., 2019) (Appendix III - Clinical assessments forms).

Figure 2.5. A diagram of the course for the Six Spot Step Test.

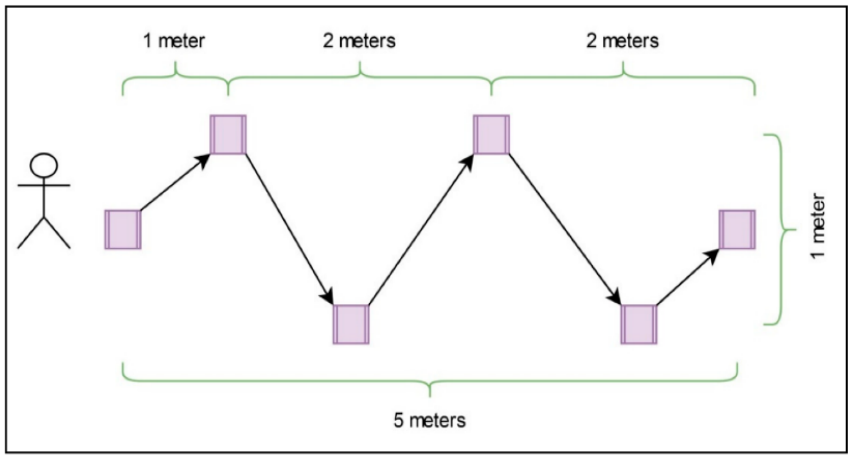


Figure 2.5: A 5 X 1-meter marked-up rectangle course in which the cubes are placed in specific positions to perform the test.

Action Research Arm Test

It is a 19-item observational measure used by physiotherapists and other health care professionals to examine upper limb performance (i.e., coordination, dexterity and functioning) (Carpinella et al., 2014). Items covering the Action Research Arm Test are categorized into four subscales (grasp, grip, pinch and gross movement) and arranged in order of decreasing difficulty, with the most difficult task examined first, followed by the least difficult task. Participants were sitting comfortable in front of a stable desk (Figure 2.6) performing each task and the performance was rated on a four-point scale, ranging from 0 (no movement) to 3 (movement performed normally).

According to the guidelines, each participant if the maximum score (score = 3) is obtained for the most difficult task then the maximum score was assigned for this subscale. If the participant was unable to complete the most difficult task (score = 0 – 2), then the easiest task in this subscale had to be accomplished. However, if the participant completely failed (score = 0) to perform the easiest item, then the other intermediate items were not be tested, and the total subscale scored as zero, and the assessor moved to the next subscale. Nevertheless, if the participant succeeded at the easiest item (completely; score = 3 or partially; score = 1 or 2), then all the other tasks in that specific subscale were tested before moving to the next subscale. Therefore, for each participant we recorded the total score for each upper limb separately as the final test result (Appendix III - Clinical assessments forms).

Figure 2.6. The Action Research Arm Test toolkit.

Figure 2.6: The Action Research Arm Test toolkit includes wooden blocks of various sizes, cricket ball, sharpening stone, alloy tubes, washer and bolt, two glasses, sharpening stone, marbles, ball bearings and a tin lid.

Muscle strength

We assessed the muscle strength of major muscle groups with the use of the Hand-Held Dynamometer (Kinvent Biomechanique, Montpelier, France), which is a dynamometer used in the evaluation and rehabilitation of isometric muscle strength that provides real time biofeedback (Andrews et al., 1996). The patient lied (supine or prone) on a therapeutic bed and the physiotherapist, with the use of the muscle controller, held against the patient's limb as the patient exerted a maximal force (Figure 2.7). The physiotherapist countered the force (make test) or tried to break the contraction (break test) and the data were stored using the KFORCE APP (Kinvent Biomechanique, Montpelier, France). Shoulder flexors, extensors, rotators, horizontal adductors and abductors, elbow flexors and extensors are the major muscle groups which were evaluated. A separate value for each muscle group was recorded to be used in the data analysis (Appendix III - Clinical assessments forms).

Figure 2.7. The Hand-Held Dynamometer assessment.

Figure 2.7: The Hand-Held Dynamometer allows the assessor to quantify the force applied during manual muscle strength testing, in a given body part position.

Symbol Digit Modalities Test.

It is a commonly used test in pwMS (Benedict et al., 2020), which measures cognitive processing speed as well as motor speed. We employed the oral form of the test, in which participants were provided the test sheet with nine symbols, each paired with a number on top of the page, defined as the "key". For example, the symbol "O" is matched with

the number “6”, so the correct response would be to say “six”. The rest of the page consists of a randomized, sequential variety of these symbols. Participants were asked to verbally respond with the number that corresponds with each symbol. During the test, the participant was given two minutes to orally match symbols with digits as quickly as possible. The score was obtained by subtracting the number of errors from the number of items completed. To diminish the practice effect, we created six different tests, as many as our assessment points, in which we rearranged the key (Roar et al., 2016) (Appendix III - Clinical assessments forms).

Modified Fatigue Impact Scale.

It is a short questionnaire which requires the participants to describe the effects of fatigue during the past four weeks (Fisk, Ritvo, et al., 1994). The Modified Fatigue Impact Scale consists of 21 questions which are subjectively rated from “0” (low rate) to “4” (high rate) and it is also divided into three subscales (i.e., physical, cognitive, and psychosocial). We recorded the total score of the test as the final test result. The higher the score is, the greater is the impact of fatigue in individual daily life. Therefore, we used the Modified Fatigue Impact Scale as the description of participants’ attribution of functional restrictions to fatigue symptoms (Appendix III - Clinical assessments forms).

Medical Outcomes Study Questionnaire Short Form 36 Health Survey.

It is a patient-reported questionnaire which is commonly used for measuring QoL (Lins & Carvalho, 2016). The Medical Outcomes Study Questionnaire Short Form 36 Health Survey evaluates eight health concepts; 1) limitations in physical activities, 2) limitations in role activities due to physical restrictions, 3) limitations in role activities due to physical restrictions, 4) general mental health, 5) bodily pain, 6) limitations in social activities, 7) fatigue and 8) general health perceptions. The specific test consists from 11 questions which are subjectively rated. The lower the score the more disability, whereas the higher the score the less disability (Appendix III_ Clinical assessments forms).

2.1.4 Analysis plan

To investigate possible effects of the exercise protocol the recommended guidelines were followed, in which a separate analysis for each of the outcome measures, in all experimental phases (i.e., baseline, intervention and follow-up) was performed. Firstly a

visual analysis was performed, to determine whether there was a functional relationship between the intervention and the outcome measures. Then, a statistical analysis was performed in all outcome measures, which indicated a sizeable effect from the visual analysis, to evaluate the magnitude of the intervention effect (Lobo et al., 2017).

Transcranial Magnetic Stimulation measures analysis

Corticospinal plasticity was determined through changes of the corticospinal excitability measures. Hence, bilateral rMT, MEPs amplitude and latency, and CMCT were quantified, because each measure can assess different plastic changes across the neuromotor axis and they can be used as a proxy of corticospinal plasticity. rMT (%MSO) is the lowest intensity needed to elicit MEPs from a single-pulse TMS, amplitude (mV) is the difference in voltage between the maximal negative to maximal positive deflection of MEPs, which is referred as peak-to-peak amplitude (Groppa et al., 2012), latency (ms) is the time between the TMS onset and the MEPs onset (Udupa & Chen, 2013), while CMCT (ms) estimates the conduction time of corticospinal fibers between motor cortex and alpha-motoneurons (Chen et al., 2008).

For both upper limbs, corticospinal excitability measures (i.e., MEPs amplitude and latency) were first calculated from each MEP trace and then the mean value was calculated to get a single value. These calculations were done according to the different time points for each participant in the baseline phase, at five time points in the intervention phase and at three time points in the follow-up phase. To investigate possible changes in corticospinal excitability, we measured rMT and calculated peak-to-peak amplitude throughout assessing MEPs (Vanteemar S. Sreeraj et al., 2019) of the Abductor Pollicis Brevis, while measuring of latency indicated possible changes in CMCT. Any changes in all measures across time points, indicated alterations in corticospinal plasticity (Paulus et al., 2013). rMT was evaluated using MTAT 2.0 (Awiszus, 2003) (available at http://clinicalresearcher.org/software.htm) and to investigate possible changes in individual corticospinal plasticity of each participant, bilaterally the difference between the mean values of each phase was calculated (Balloff et al., 2022; Stampanoni Bassi, Buttari, et al., 2020). On the other hand, from each stimulus response during the suprathreshold stimulation (i.e., 120% of rMT) (Paulus et al., 2013), MEPs peak-to-peak amplitude and latency were calculated, offline. To define CMCT (ms), the peripheral conduction time ((*F*-wave latency + *M*-wave latency – 1)/2) is subtracted from the MEPs

latency. *F*-wave is the response of the targeted muscle produced by antidromic activation of motoneurons following the peripheral stimulation of motor nerve fibers, whereas *M*-wave produced by the direct muscle response (Fisher, 2007; Hallett, 2007; McNeil et al., 2013). A prolonged CMCT indicates damage of the myelinated axons, demyelination of central motor pathways or slow summation of descending excitatory potentials in the corticospinal tract evoked by TMS (Hallett, 2007; Zimnowodzki et al., 2020). To standardize the latencies of all motor responses derived from different stimulation protocols (i.e., MEPs, *F*- and *M*- wave), a visual inspection from stimulation onset to response onset was used, performed from the same investigator so to ensure reliability and reproducibility of these measures across all time points. To define possible changes in CMCT, the difference between the mean values of each phase bilaterally was evaluated.

Clinical measures analysis

For each clinical measure (i.e., balance, gait, cognitive function, bilateral hand dexterity, strength, Modified Fatigue Impact scale, Medical Outcomes Study Questionnaire Short Form 36 Health Survey) the mean values from each time point across all phases (i.e., baseline, intervention, follow-up) were calculated, so to get a single mean value for each measure and for each phase (i.e., mean baseline, mean intervention, mean follow-up). To investigate the effect of the intervention protocol on the clinical condition, the differences between phases' mean values (i.e., mean baseline, mean intervention, mean follow up) were calculated, reflecting to the degree of the intervention-elicited change on the clinical condition following in-phase bilateral exercises.

Visual analysis

Initially, a visual analysis was conducted and data is presented graphically in spaghetti plots, to define whether there is a functional relation between the intervention and the outcome measures (Lobo et al., 2017). During the visual analysis, six features of the research design graphed data was examined: level, trend, stability, immediacy of the effect, overlap, and consistency. Over the within-phase examination an evaluation of level, trend and stability were examined. Level was reported from the mean score of each dependent variable and trend was determined as whether the data points are monotonically decreased or increased. To quantify the within phase differences in level and thus to identify whether there is substantial increase in the targeted behaviors, the

Percentage of data Exceeding the Median (PEM) method (MA, 2006) was used. Stability was estimated based on the percentage of data points falling within 15% of the phase median, if this was higher than 80% then was assumed that this criterion was met. Additionally, over the between-phase examination an evaluation of overlapping data among baseline and intervention phases, consistency of data patterns and immediacy of effect were performed (Lobo et al., 2017). Immediacy of the effect was examined by comparing changes in level between the last three data points of one phase (e.g., baseline) and the first three data points of the next phase (e.g., intervention). Furthermore, consistency of data patterns involved the observation of the data from all phases within the same condition, with greater consistency expressing greater causal relation. Each feature was assessed individually and collectively across to all participants and all phases. Consequently, if the intervention protocol was the sole determinant of improvement, indicators of improvement only at the intervention phase, were expected to be found.

Additionally, two independent assessors were systematically measured each outcome measure across time and inter-assessor agreement was calculated on at least twenty percent of the data points in each condition. The minimum acceptable inter-assessor agreement was set to 0.8 (Kratochwill et al., 2010)

Statistical analysis

A statistical analysis was performed only for the concurrent multiple baseline design study, since the single case study was a pilot study and a comprehensive statistical analysis was unable to conducted. The limited sample size was insufficient to yield statistically significant conclusions.

A visual analysis was performed for each of the outcome variables to test for any effects due to the intervention. If the visual analysis indicated potential functional effects and met the six features (i.e., level, trend, stability, immediacy of the effect, overlap, and consistency) between baseline and intervention phase, the Nonoverlap of All Pairs (NAP) metric was used in order to estimate the effect of the intervention and randomization tests were constructed to evaluate statistical significance (Krasny-Pacini & Evans, 2018; Lobo et al., 2017; Zimnowodzki et al., 2020).

The null hypothesis was that there would be no improvement from the intervention protocol, thus participants' responses are independent from the condition (baseline vs.

intervention) under which they were observed. The alternative hypothesis was that the neurophysiological parameters and/or the clinical condition of the participants would be affected by the specific intervention, assessed separately. The null hypothesis was rejected if the *p* – value was smaller than the Bonferroni corrected *p* – value based on the actual number of tests that were performed (0.05/number of tests). All tests were two sided. Statistical analysis was performed using the statistical software R (https://www.r-project.org/).

2.2 Single Case Pilot Study Design

The specific participant was recruited and evaluated by a neurologist at The Cyprus Institute of Neurology and Genetics on June 2022 and participated in the study from 4th to 29th of July 2022. The participant was recruited following the inclusion criteria, who completed all assessments and the exercise protocol without complaints or side effects. Demographics and baseline clinical characteristics of the participant was collected prior to the intervention and are presented in Table 2.1.

Table 2.1. Participant's demographic and baseline clinical characteristics – single case design.

Age (years)	35
Sex	Female
Dominant hand	Right
Weight (kg)	57
Height (m)	1.59
BMI	22.5 (normal)
HR[a] max (bpm)	185
Left upper limb length[b] (cm)	60
Right upper limb length[b] (cm)	60
EDSS	2
Disease duration (years)	4

Current clinical symptoms	- General weakness. - Minor imbalance during gait.
MMSE (Folstein et al., 1975)	30 (no cognitive impairment)
MS related medication	Gilenya (Kappos et al., 2015; Zécri, 2016)
Occupation	Sedentary

Table 2.1: BMI; Body Mass Index, HR; Heart Rate, EDSS; Expanded Disability Status Scale, MMSE; Mini Mental State Examination, MS; Multiple Sclerosis [a]; Heart Rate maximum was calculated from the equation 220-age (Tanaka et al., 2001a), [b]; Upper limbs length were measured in an anatomical position from C7 spinous process to the ulnar head (Livingston et al., 2010). Because upper limb length is a factor contributing to MEPs responses (Livingston et al., 2013), both upper limbs' length was measured, without a difference between both sides. EDSS score was two, which indicates minimal disability (Kurtzke, 1983). The Mini Mental State Examination and current clinical symptoms were in line with inclusion criteria, so there was no specific impact related to the TMS and clinical measures. The participant was engaged in sedentary work (Thivel et al., 2018; Tremblay et al., 2017), which required low physical demands and no frequent moves; thus, it may not have an impact on the study's outcome measures.

This pilot study followed a single case study design without blinding and included baseline and intervention phases (Figure 2.8). Several TMS-based neurophysiological measures and clinical assessments (see outcome measures) were performed.

Figure 2.8. Timeline and schematic representation of the pilot study's design.

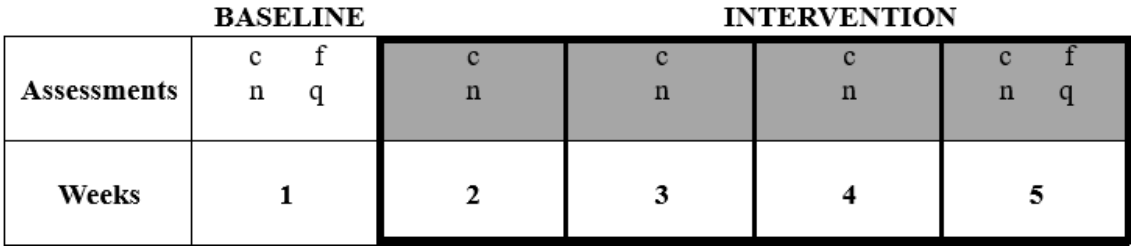


Figure 2.8: c; clinical assessment, n; neurophysiological assessment via TMS, f; Modified Fatigue Impact Scale questionnaire, q; Medical Outcomes Study Questionnaire Short Form 36 Health Survey. Grey colour represents the intervention phase. The first row represents the participant's data points . The second row represents the different weeks, so every procedure which was included (i.e., c, n, f, q), it was performed during the corresponding week but in different days.

Baseline

As depicted in Figure 2.8, the patient the patient began the study with the baseline phase, one week prior to the intervention phase. At the baseline phase, the participant performed the neurophysiological examinations (i.e., CMCT, rMT, MEPs amplitude and latency), the clinical assessments (i.e., gait, balance, strength, hand dexterity, cognitive functions) and completed the Modified Fatigue Impact Scale (Fisk, Ritvo, et al., 1994) and Medical Outcomes Study Questionnaire Short Form 36 Health Survey (Lins & Carvalho, 2016).

Intervention

The intervention protocol consisted of exercises based on in-phase bilateral movements of the upper limbs, which were adapted to different sport activities and to fitness functional exercises (see page 80), organized in a circuit training considering the MS exercise recommendations (Kalb et al., 2020). According to the American College of Sports and Medicine guidelines and based on the review of Kalb at al. (2020), (Table 2.2), pwMS should be encouraged to exercise at least 150min per week (Kalb et al., 2020).

The exercise frequency ranges between two and three days per week, which included different types of exercise that focuses on large muscle groups and adequate rest between sets. However, MS-specific characteristics and safety should be considered during prescription of an exercise protocol, which must include appropriate modifications and gradual progression. For the needs of the current study, both groups exercised in a cool environment (i.e., 24°C) which minimizes the risk of fatigue or hyperthermia, allowing individuals with MS to exercise safely and effectively.

Table 2.2. Exercise recommendations for people with Multiple Sclerosis.

Recommended exercise	
EDSS 0 – 4.5 (mild impairments)	
Aerobic	- 2 – 3x/week - 10 – 30 minutes at a moderate exercise intensity (40%–60% of maximum HR or aerobic capacity) - 3 – 4 RPE (on a 10-point RPE) - Cycle ergometer (upper limbs, lower limbs or combined) - Treadmill or overground walking

	- Sport activities - Aquatic therapy - Group-based exercises (target to individual limitations).
Advanced aerobic	- 5x/week - up to 40 minutes - 70% of peak aerobic capacity or 80% of maximum HR - Up to 7 RPE (on a 10-point RPE) - Cycle ergometer (upper limbs, lower limbs or combined) - Treadmill or overground walking - Sport activities, Aquatic therapy, Running, road cycling, walking - Group-based exercises (target to individual limitations).
Resistance	- 2 – 3x/week, 1–3 sets for each exercise, 8–15 repetitions/set, 5 – 10 exercises - Body weight exercises, free weights, resistance bands - Close-chain exercises are preferred.
Flexibility	- Daily, 2 – 3 sets of each stretch - Static stretching, yoga, clinical Pilates. - Group-based exercises (target to individual limitations).
Neuromotor	- 3 – 6x/week, 20 – 60 minutes - Interventions individualized for intensity and duration, targeting fall prevention, postural stability, coordination - Dance therapy, clinical yoga, Tai chi, hippotherapy, virtual reality, balance and motor control training, Sport activities, Aquatic therapy, group-based activities.
EDSS 5 – 6.5 (increasing mobility impairments)	
Aerobic Resistance Flexibility Neuromotor	Same as EDSS 0 – 4.5
Adaptive exercise	- 3 – 6 RPE (on a 10-point RPE) - Recumbent hand-cycle or three-wheel bike for cycling, pole-walking

	- Aerobic: heat sensitivity in some patients may require cooling interventions - Resistance: functional/multi-joint movements (sit-to-stand, stair climbing, reaching); neuromuscular electrical stimulation.

Table 2.2: EDSS; Expanded Disability Status Scale, HR; Heart Rate, RPE; Rate of Perceived Exertion. According to the individual EDSS score, specific guidelines related to different types of exercises are recommended to be performed either from health professionals, either form people with Multiple Sclerosis.

Since no established protocols have been previously reported, for the needs of the current study a certified fitness instructor designed these protocols adapted to different sport activities and fitness exercises. Specifically, each session consisted of one to three sets, consisting of 20 – 30 repetitions of 9 different exercises targeting large muscle groups of the upper limbs (shoulder flexors, extensors, rotators, abductors and adductors, elbow flexors and extensors, hand and finger flexors and extensors). Additionally, three exercises targeted large lower limbs muscle groups (hip flexors, extensors, abductors and adductors, knee and ankle flexors and extensors) which were performed in between the upper limbs' exercises and allowed relaxation of the upper limbs' muscles.

The specific exercises included sports activities of basic technical skills of basketball (e.g., different types of passing, catching and throwing the ball) and volleyball (e.g., different types of passing and receiving the ball), whereas the fitness exercises included the diagonal movements from proprioceptive neuromuscular facilitation technique (PNF) (Surburg & Schrader, 1997), as well as fingers flexion and extension by the use of a resistance hand training net (Akbar et al., 2020). All exercises are presented in Appendix IV - Examples of the exercise protocol**.**

The intervention phase consisted of four consecutive weeks in which the protocol was performed three times per week, for 30 – 60 minutes each session, adapted to the participant's fatigue and fitness level. Every intervention session consisted of a five-minute warm-up (i.e., whole body range of motion exercises), followed by the main sport activities and fitness exercise protocol as described above and a cool down for five minutes (i.e., passive stretching exercises of the muscle groups which are involved in the main part).

Additionally, four neurophysiological and four clinical assessments (i.e., once a week) were performed, to collect four data points across the intervention phase. Moreover, the participant was asked to complete the Modified Fatigue Impact Scale and the Medical Outcomes Study Questionnaire Short Form 36 Health Survey (see secondary measures), once, at the end of the intervention phase (Figure 2.8) (Akbar et al., 2020).

The exercise protocol lasted four consecutive weeks and contained three sets of 12 exercises within each set, with two minutes rest between the sets. All details regarding the exercise protocol are presented in Table 2.3.

Table 2.3. Overview of the exercise protocol – single case design.

	Type of exercise	Repetitions	Body position	Difficulty level
1	Basketball chest pass	20-30	Standing	Distance of the pass
2	PNF 1st diagonal FP	20-30	Standing	Elastic band
3	Flexion of all fingers	20-30	Sitting	Hand training net
4	Adductors squeeze	20-30	Supine lying	Pilates ring
5	Basketball shoulder pass	20-30	Standing	Distance of the pass
6	PNF 1st diagonal EP	20-30	Standing	Elastic band
7	Extension of all fingers	20-30	Sitting	Hand training net
8	Hips Abduction	20-30	Supine lying	Pilates ring
9	Volleyball overhead pass	20-30	Standing	Distance of the pass
10	PNF 2nd diagonal FP	20-30	Standing	Elastic band
11	PNF 2nd diagonal EP	20-30	Standing	Elastic band
12	Squat	20-30	Standing	Balance pads

Table 2.3: PNF; Proprioceptive Neuromuscular Facilitation, FP; Flexion Pattern, EP; Extension Pattern. Each session included three sets of nine different exercises which targeted large muscle groups of the upper limbs (i.e., 1-3, 5-7, 9-11) and three exercises which targeted large muscle

groups of the lower limbs (i.e., 4, 8, 12). The range of repetitions was 20 – 30 according to participant's fitness level. The difficulty level for exercises 4 and 8 was maintained by changing the resistance of the Pilates ring, whereas for exercise 12 the difficulty level was sustained by changing the base of support (e.g., balance pads, Bosu ball). The difficulty level for the sport activities (i.e., 1, 5, 9) was maintained by changing the distance of the passes. All PNF exercises (i.e., 2, 6, 10, 11) were performed against the resistance of elastic bands (different resistance accordingly), which it was attached by a stable point.

For the duration of the intervention implementation period, there was a continuous monitoring and record of the participant's performance. Individual performance data are presented in Table 2.4.

Table 2.4. Individual performance during the exercise protocol.

Sessions Completed	12/12
Number of Repetitions	716
HR (bpm)	101
%HR maximum	62
RPE	5
Body Temperature **(**℃)	36
Resistance Level	Medium

Table 2.4: HR; Heart Rate RPE; Rating of Perceived Exertion (Foster et al., 2001). The participant completed all the exercise sessions and she exceeded the recommended number (i.e., 300) of repetitions required in a session to induce neuroplastic effects (Catherine et al., 2016). Additionally, the participant completed the exercise protocol following the recommended exercise features regarding HR, RPE, body temperature and resistance level (Kalb et al., 2020). However, the percentage of the maximum HR indicated that the participant did not exceeded the aerobic level of exercise (i.e., below 70% of each participant's maximum HR) (Skinner & McLellan, 1980) during the sessions.

Considering that the individual HR should be within the range of 50 – 70% of the individual maximum HR, the progression and difficulty level were gradually enhanced. The progression was managed by increasing the distance of the passes and the resistance of the elastic bands according to each exercise. This approach ensured that the exercises

remained challenging yet achievable, maximizing the benefits for participants without risking overexertion or exceeding their individual aerobic capacity.

Since the main aim was to induce neuroplasticity as an effect of the specific type of movement (i.e., in-phase bilateral movement of the upper limbs) and not as a training effect, (Diechmann et al., 2021; Hortobágyi et al., 2021; Learmonth & Motl, 2021; Pickersgill et al., 2022) participant's performance was maintained under the HR zone of aerobic exercise (i.e., below 70% of the individual maximum HR (Skinner & McLellan, 1980). To keep a constant individual HR and body temperature, at the end of each set across sessions, a pulse oximeter (ChoiceMMed OxyWatch C29, Bristol, United States) for the HR screening was used and a forehead thermometer was employed to monitor body temperature.

Throughout the literature, aerobic exercise at 70% of an individual's maximum HR is considered moderate to vigorous intensity and is particularly effective for promoting neuroplasticity (Cotman et al., 2007; Maass et al., 2015; Skinner & McLellan, 1980). This intensity level enhances the release of brain-derived neurotrophic factor, a key protein that supports the survival and growth of neurons and synapses. Also, exercise at 70% intensity increases cerebral blood flow, delivering oxygen and nutrients to brain tissues, which further supports neuronal health and plasticity. Over time, regular engagement in such aerobic activity has been associated with reduced risks of neurodegenerative diseases, enhanced emotional regulation, and improved recovery from brain injuries (Cardoso et al., 2024; Devasahayam et al., 2017; Diechmann et al., 2021).

Additionally, a forehead thermometer was used to monitor the body temperature, as well as the room temperature was controlled at 24℃, so the participant was always exercising at the same temperature. It is well established that pwMS are particularly sensitive to changes in body and environmental temperature, a phenomenon known as Uhthoff's phenomenon (Frohman et al., 2013). Elevated core body temperature, often caused by exercise, fever, or hot environments, exacerbates symptoms such as fatigue, weakness, and cognitive dysfunction. This temperature sensitivity occurs because increased heat impairs the conduction of already damaged axons in demyelinated regions of the nervous system.

Exercise, although beneficial for managing MS symptoms and enhancing QoL, presents unique challenges under temperature extremes. High environmental temperatures can

reduce exercise tolerance and increase symptom severity, while colder conditions may improve exercise performance but can lead to muscle stiffness or spasticity in some cases (Chacko et al., 2021; Christogianni et al., 2018; Romberg et al., 2012). Understanding and managing the interplay between temperature and MS symptoms is essential for promoting safe and effective exercise participation in this population. Therefore, cooling strategies, such as pre-cooling or exercising in a climate-controlled environment, can mitigate these effects and improve performance (Christogianni et al., 2018; Davis et al., 2010; Stevens et al., 2023).

2.2.1 Results - Primary outcome measure

Central Motor Conduction Time

A decrease in CMCT signifies an improvement. The results of the CMCT assessments during all time points are depicted in Figure 2.9 and they are also presented in Appendix V - Single Case Pilot Study Design – Results; Table 1.

Figure 2.9. Visual representation of the Central Motor Conduction Time during baseline and intervention phases – single case pilot study.

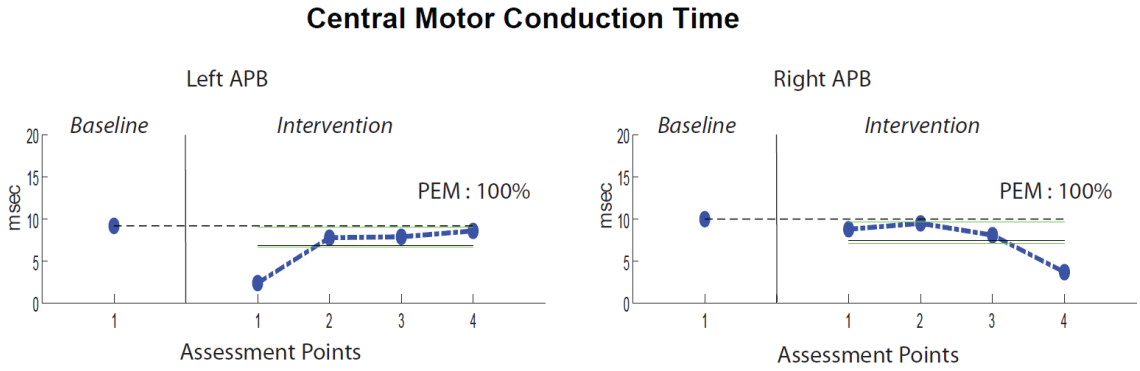


Figure 2.9: APB; Abductor Pollicis Brevis, PEM; Percentage Exceeding the Median. Data of the participant are presented regarding left and right upper limb, in terms of left and right APB. The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the values of the central motor conduction time are presented. Central motor conduction time, is measured by means of milliseconds (msec). The vertical lines between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines, refers to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases. The black horizontal lines represent the

within-phase (i.e., intervention) mean. Although, the mean lines may not be clearly visible as they are superimposed with the green lines.

Within-phase visual analysis: Data for both left and right upper limbs were analyzed in terms of variability (i.e., the spread of data points within a phase that was indicated from the two-standard deviation band, which is the mean of a phase and adding and subtracting two standard deviations from it (Krasny-Pacini & Evans, 2018), stability (Lobo et al., 2017) and trend. Low variability (i.e., 0 – 20%) was observed during the intervention phase for both upper limbs. However, no stability of the data (>80% (Lobo et al., 2017)) for both upper limbs was observed during the intervention phase (75%). In terms of the trends of the CMCT, during the intervention phase the participant showed an upward trend for the left upper limb, whereas a downward trend was observed for the right upper limb. As a conclusion, the participant did not meet all the within-phase visual analysis' criteria, for either upper limb.

Between-phases visual analysis: Data from both baseline and intervention phases were included in the between-phases analysis. The criteria which were used for the visual analysis were level (i.e., change of mean values between phases), immediacy (i.e., change in level between the data point of the baseline phases and the data points of the intervention phases) and the PEM (MA, 2006). Reduction (i.e., improvement) of the mean value was observed, which it is interpreted as a difference in level and immediate effect for both upper limbs. The PEM (MA, 2006) indicates an effect when 70% of data of the intervention phase exceed the median of the baseline phase. According to our results, the participant showed an effect for both upper limbs (PEM=100%).

In summary, the participant did not meet all visual analysis' criteria (Lobo et al., 2017).

2.2.2 Results - Secondary outcome measures

Motor Evoked Potentials Amplitude

An increase in MEPs amplitude signifies an improvement. Throughout the within and between-phases visual analysis, no evidence of improvement related to MEPs amplitude was observed. The results of the MEPs amplitude assessments during all time points are depicted in Figure 2.10 and they are also presented in Appendix V - Single Case Pilot Study Design – Results; Table 2.

Figure 2.10. Visual representation of the Motor Evoked Potentials Amplitude during baseline and intervention phases – single case pilot study.

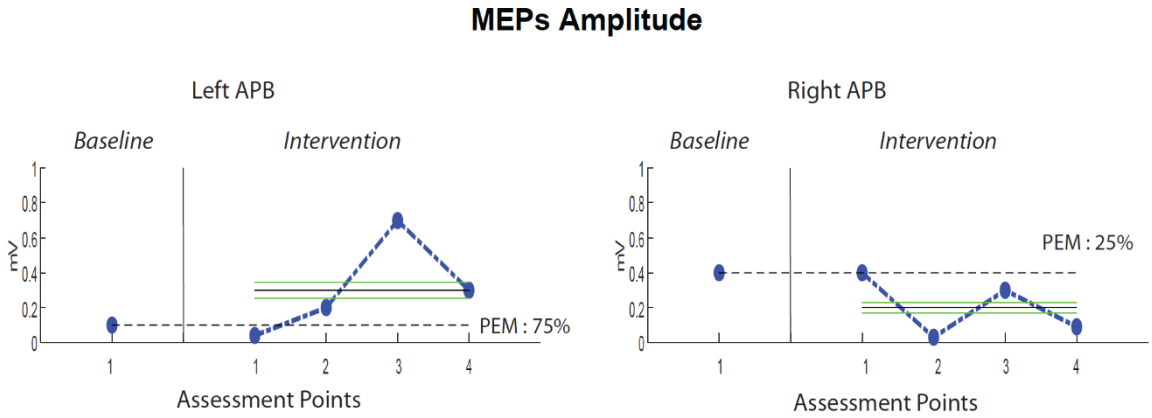


Figure 2.10: MEPs; Motor Evoked Potentials, APB; Abductor Pollicis Brevis, PEM; Percentage Exceeding the Median. Data of the participant are presented regarding left and right upper limb, in terms of left and right APB. The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the values of the MEPs amplitude are presented. MEPs amplitude, is measured by means of millivolts (mV). The vertical lines between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines, refers to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases. The black horizontal lines represent the within-phase (i.e., intervention) mean. Throughout the within phase (i.e., intervention) visual analysis, data variability and instability could be observed for both upper limbs.

Within-phase visual analysis: Data for both left and right upper limbs were analyzed in terms of variability (i.e., the spread of data points within a phase that was indicated from the two-standard deviation band, which is the mean of a phase and adding and subtracting two standard deviations from (Krasny-Pacini & Evans, 2018), stability (Lobo et al., 2017) and trend. Low variability (i.e., 0 – 20%), was observed during the intervention phase for both upper limbs. However, no stability of the data (>80% (Lobo et al., 2017)) for both upper limbs was observed during the intervention phase (75%). In terms of the trends of the CMCT, during the intervention phase the participant showed an upward trend for the left upper limb, whereas a downward trend was observed for the right upper limb. As a conclusion, the participant did not meet all the within-phase visual analysis' criteria, for either upper limb.

Between-phases visual analysis: Data from both baseline and intervention phases were included in the between-phases analysis. The criteria which were used for the visual analysis, were level (i.e., change of mean values between phases), immediacy (i.e., change in level between the data point of the baseline phases and the data points of the intervention phases) and the PEM (MA, 2006). Reduction (i.e., improvement) of mean values was observed, which it is interpreted as a difference in level and immediate effect for the left upper limb. On the other hand, increase (i.e., no improvement) of mean values was observed for the right upper limb, which it is interpreted as no difference in level and no effect for that specific upper limb. The PEM (MA, 2006) indicates an effect when 70% of data of the intervention phase exceed the median of the baseline phase. According to our results, the participant showed an effect only for the left upper limbs (PEM=75%) (Figure 2.10).

In summary, the participant did not meet all visual analysis' criteria for either upper limb (Lobo et al., 2017).

Motor Evoked Potentials Latency

Reduction in MEPs latency signifies an improvement. Throughout the within and between-phases visual analysis, no evidence of improvement related to MEPs latency was observed. The results of the MEPs latency assessments during all time points are depicted in Figure 2.11 and they are also presented in Appendix V - Single Case Pilot Study Design – Results; Table 3.

Figure 2.11. Visual representation of the Motor Evoked Potentials Latency during baseline and intervention phases – single case pilot study.

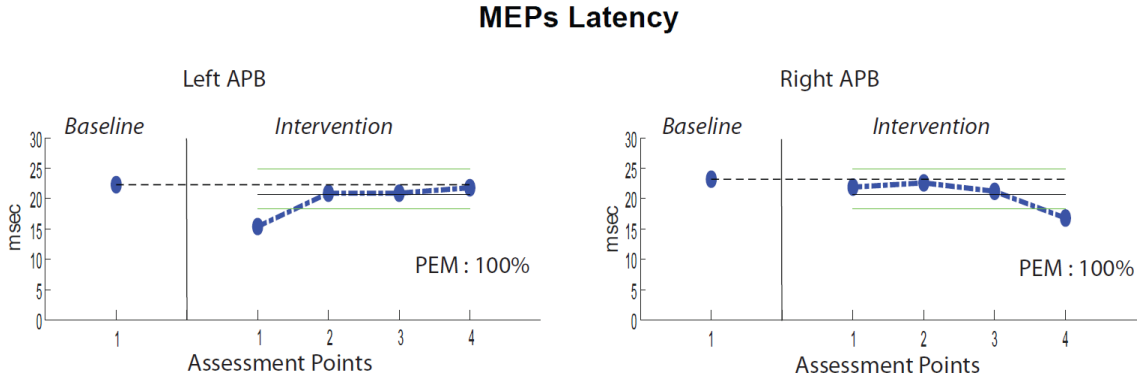


Figure 2.11: MEP; Motor Evoked Potentials, APB; Abductor Pollicis Brevis, PEM; Percentage Exceeding the Median. Data of the participant are presented regarding left and right upper limb, in terms of left and right APB. The number of assessment points per phase are presented on the

x-axis, whereas on the y-axis the values of the MEPs latency are presented. MEPs latency, is measured by means of milliseconds (msec). The vertical lines between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines, refers to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Within-phase visual analysis: Data for both left and right upper limbs were analyzed in terms of variability (i.e., the spread of data points within a phase that was indicated from the two-standard deviation band, which is the mean of a phase and adding and subtracting two standard deviations from it (Krasny-Pacini & Evans, 2018), stability (Lobo et al., 2017) and trend. Low variability (i.e., 0 – 20%), was observed during the intervention phase for both upper limbs. However, no stability of the data (>80 Between-phases visual analysis: Data from both baseline and intervention phases were included in the between-phases analysis. The criteria which were used for the visual analysis, were level (i.e., change of mean values between phases), immediacy (i.e., change in level between the data point of the baseline phases and the data points of the intervention phases) and the PEM (MA, 2006). Reduction (i.e., improvement) of mean values was observed which it is interpreted as a difference in level and immediate effect for the both upper limbs. The PEM (MA, 2006) indicates an effect when 70% of data of the intervention phase exceed the median of the baseline phase. According to our results, the participant showed an effect for both upper limbs (PEM=100%).

In summary, the participant did not meet all visual analysis' criteria for both upper limbs was observed during the intervention phase (75%). In terms of the trends of the CMCT, during the intervention phase the participant showed an upward trend for the left upper limb, whereas a downward trend was observed for the right upper limb. As a conclusion, the participant did not meet all the within-phase visual analysis' criteria, for either upper limb.

Between-phases visual analysis: Data from both baseline and intervention phases were included in the between-phases analysis. The criteria which were used for the visual analysis, were level (i.e., change of mean values between phases), immediacy (i.e., change in level between the data point of the baseline phases and the data points of the intervention phases) and the PEM (MA, 2006). Reduction (i.e., improvement) of mean values was

observed which it is interpreted as a difference in level and immediate effect for the both upper limbs. The PEM (MA, 2006) indicates an effect when 70% of data of the intervention phase exceed the median of the baseline phase. According to our results, the participant showed an effect for both upper limbs (PEM=100%).

In summary, the participant did not meet all visual analysis' criteria (Lobo et al., 2017).

Resting Motor Threshold

A decrease in rMT signifies an improvement. The repeated assessments of the rMT across all assessment time points are depicted in Figure 2.12 and they are also presented in Appendix V - Single Case Pilot Study Design – Results; Table 4.

Figure 2.12. Visual representation of the Resting Motor Threshold during baseline and intervention phases – single case pilot study.

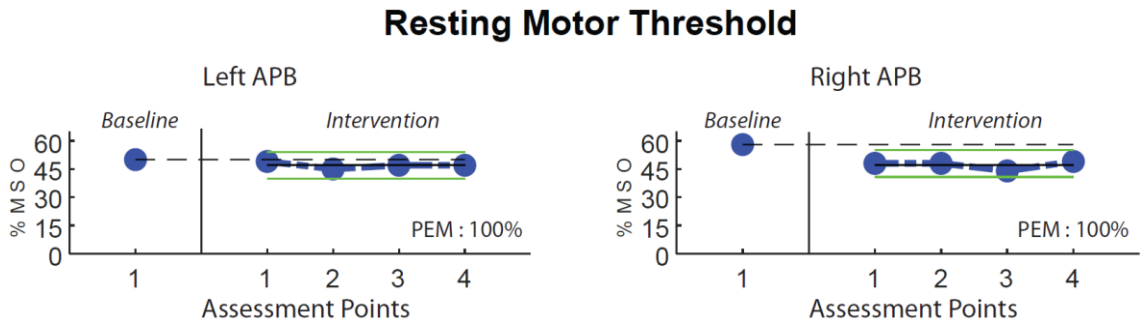


Figure 2.12: APB; Abductor Pollicis Brevis, PEM; Percentage Exceeding the Median, MSO; Maximum Stimulator Output. Data of the participant are presented regarding left and right upper limb, in terms of left and right APB. The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the values of the resting motor threshold are presented. Resting motor threshold, is measured by means of the percentage of the MSO. The vertical lines between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Within-phase visual analysis: Data for both left and right upper limbs were analyzed in terms of variability (i.e., the spread of data points within a phase that was indicated from the two-standard deviation band, which is the mean of a phase and adding and subtracting two standard deviations from it (Krasny-Pacini & Evans, 2018), stability (Lobo et al., 2017) and trend. Low variability (i.e., 0 – 20%), was observed during the intervention

phase for both upper limbs. Also, stability of the data (>80% (Lobo et al., 2017)) for both upper limbs was observed during the intervention phase (100%). In terms of the trends of the rMT, although during the intervention phase the participant showed a stable trend (i.e., expected direction) for both upper limbs, a difference in level was observed as well which indicates an improvement of the rMT. As a conclusion, the participant met all the within-phase visual analysis' criteria, for both upper limbs.

Between-phases visual analysis: Data from both baseline and intervention phases were included in the between-phases analysis. The criteria which were used for the visual analysis, were level (i.e., change of mean values between phases), immediacy (i.e., change in level between the data point of the baseline phases and the data points of the intervention phases) and the PEM (MA, 2006). Reduction (i.e., improvement) of the mean was observed which it is interpreted as a difference in level and immediate effect for both upper limbs value. The PEM (MA, 2006) indicates an effect when 70% of data of the intervention phase exceed the median of the baseline phase. According to our results, the participant showed an effect for both upper limbs (PEM=100%). Consequently, the participant met all visual analysis' criteria (Lobo et al., 2017).

Clinical Assessment

The analysis indicated an improvement on all clinical assessments (i.e., Mini-Balance Evaluation Systems Test, Six Spot Step test, Action Research Arm Test, Hand Held Dynamometer, Symbol Digit Modalities Test, Modified Fatigue Impact Scale, Medical Outcomes Study Questionnaire Short Form 36 Health Survey). All criteria of the visual analysis (Lobo et al., 2017) were satisfied for both within and between-phases analysis, for both left and right upper limbs. The repeated assessments of the previews mentioned outcome measures across all assessment time points are depicted in Figures 2.13 – 2.19 and they are also presented in Appendix V - Single Case Pilot Study Design – Results; Tables 5 – 11.

Within-phase visual analysis: Data for both left and right upper limbs were analyzed in terms of variability (Krasny-Pacini & Evans, 2018), stability (Lobo et al., 2017) and trend. The participant showed no variability (Krasny-Pacini & Evans, 2018), high data stability (100%) (Lobo et al., 2017) and expected downward trends directions during the intervention phase.

Between-phases visual analysis: Data form baseline and intervention phases were included in the between-phases analysis. The criteria used for the data analysis included level, proportion of data overlap, immediacy and the PEM (MA, 2006). The participant showed a change (i.e., improvement) in level, with no data overlapping between phases for both left and right upper limbs. Also, all participants showed an immediate effect and a high level of effectiveness (PEM = 100%) (MA, 2006), for both left and right upper limbs.

Figure 2.13. Visual representation of the Mini Balance Evaluation System Test during baseline and intervention phases – single case pilot study.

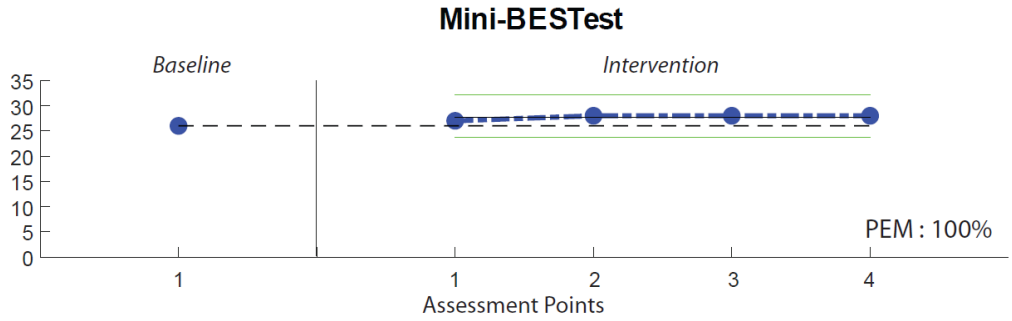


Figure 2.13: PEM; Percentage Exceeding the Median. x-axis represents the number of assessment points, while the y-axis refers the score of the tests. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Figure 2.14. Visual representation of the Six Spot Step Test during baseline and intervention phases – single case pilot study.

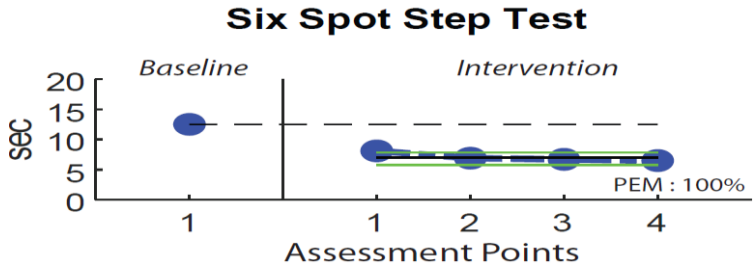


Figure 2.14: PEM; Percentage Exceeding the Median. The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the values of the test are presented. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15%

of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Figure 2.15. Visual representation of the Action Research Arm Test during baseline and intervention phases – single case pilot study.

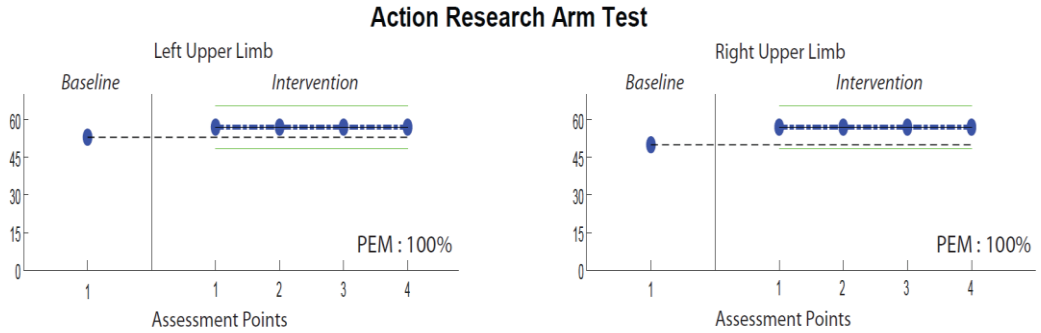


Figure 2.15: PEM; Percentage Exceeding the Median. x-axis represents the number of assessment points, while the y-axis refers the score of the tests. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Figure 2.16. Visual representation of the Hand Grip Test during baseline and intervention phases – single case pilot study.

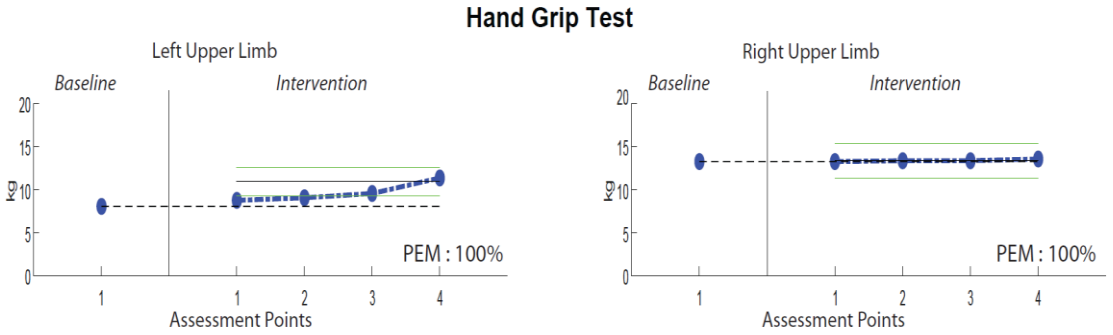


Figure 2.16: PEM; Percentage Exceeding the Median. The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the values of the muscle strength test are presented. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and

intervention phases. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Figure 2.17. Visual representation of the Symbol Digit Modalities Test during baseline and intervention phases – single case pilot study.

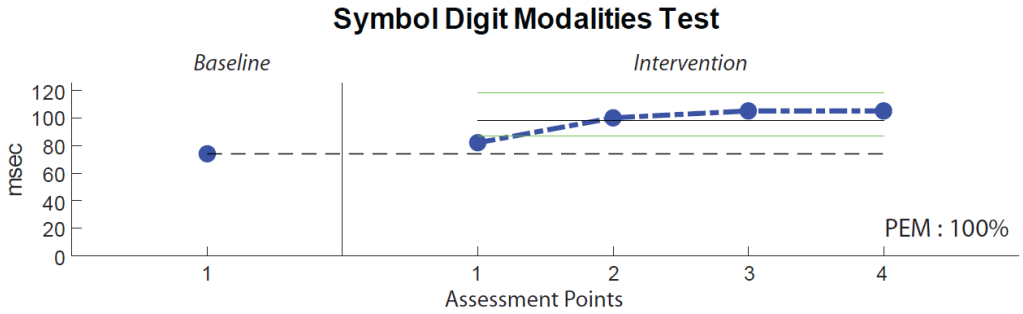


Figure 2.17: PEM; Percentage Exceeding the Median. x-axis represents the number of assessment points, while the y-axis refers the score of the tests. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Figure 2.18. Visual representation of the Modified Fatigue Impact Scale during baseline and intervention phases – single case pilot study.

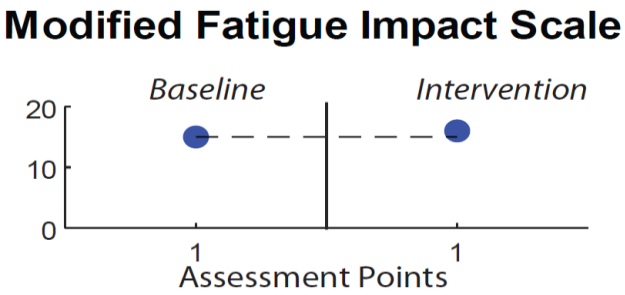


Figure 2.18*:* x-axis represents the number of assessment points, while the y-axis refers the score of the tests. The vertical line between the data points indicate the two study phases. Black horizontal dashed line refers to PEM (MA, 2006), which is calculated between phases.

Figure 2.19. Visual representation of the Medical Outcomes Study 36-items Short Form Health Survey during baseline and intervention phases – single case pilot study.

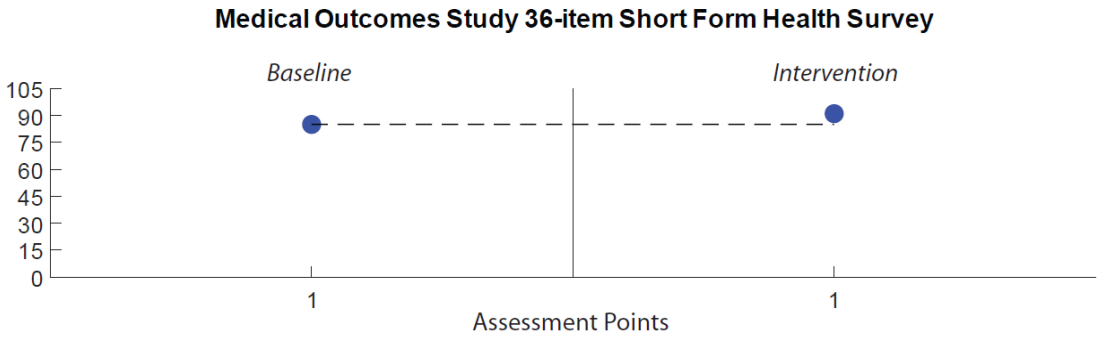


Figure 2.10: x-axis represents the number of assessment points while the y-axis refers the score of the tests. The vertical line between the data points indicate the two study phases. Black horizontal dashed line refers to PEM (MA, 2006), which is calculated between phases.

As a conclusion, according to visual analysis of CMCT, MEP amplitude, and latency measurements revealed no improvements in any participants, likely due to high variability, low data stability, and unexpected trends (upward or downward) across the two study phases. However, the intervention did have a notable effect on rMT. This suggests that while the exercises did not impact neural conduction speed, they may have increased neural excitability. Additionally, the protocol led to observable improvements in clinical outcomes, including motor function and cognitive processing.

Building on these positive findings and recognizing the limitations of the pilot study, a follow-up study with five pwRRMS was conducted, using a more refined methodology to obtain more reliable and robust results.

2.3 Multiple Baseline Study Design.

This research study has been registered as a clinical trial (ClinicalTrials.gov NCT05367947) and it was published as a registered report protocol and as a research protocol in the "PLOSONE" scientific journal.

"Sokratous, D., Charalambous, C. C., Papanicolaou, E. Z., Michailidou, K., & Konstantinou, N. (2023). Investigation of in-phase bilateral exercise effects on corticospinal plasticity in relapsing remitting multiple sclerosis: A registered report single-case concurrent multiple baseline design across five subjects. Plos one, 18(3), e0272114. https://doi.org/10.1371/journal.pone.0272114"

"Sokratous, D., Charalambous, C. C., Zamba—Papanicolaou, E., Michailidou, K., & Konstantinou, N. (2024). A 12-week in-phase bilateral upper limb exercise protocol promoted neuroplastic and clinical changes in people with relapsing remitting multiple sclerosis: A registered report randomized single-case concurrent multiple baseline study. Plos one, 19(10), e0299611. https://doi.org/10.1371/journal.pone.0299611"

All participants were recruited and evaluated by a neurologist at The Cyprus Institute of Neurology and genetics from January to February 2023, and then they randomly enrolled (Figure 2.20) and participated in the study from 10th of March to 13th of October 2023. Individual medical records were collected on 1st of March 2023.

Figure 2.20. Multiple baseline design CONSORT diagram.

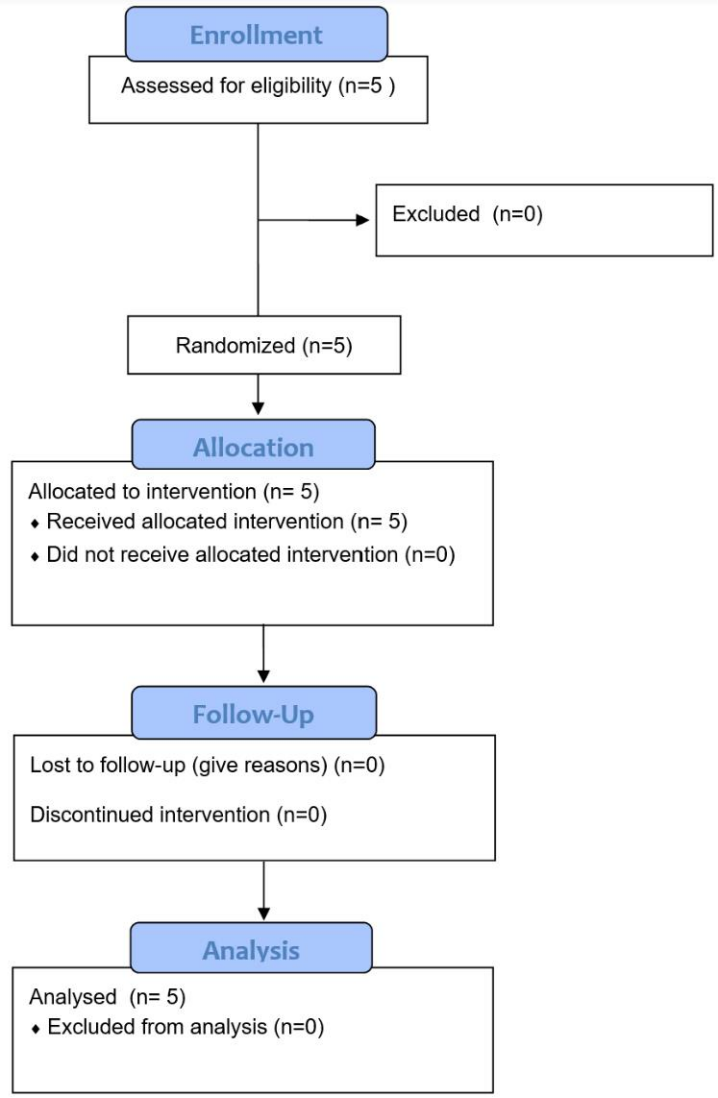


Figure 2.20: According to our study design (i.e., single case concurrent multiple baseline design across subjects), five participants were recruited and enrolled the study according to the inclusion/exclusion criteria. All of them finished the intervention protocol and all of them were included in data analysis.

The study followed a single-case concurrent multiple baseline design across five subjects, without blinding, which is based on the single case experimental design methodology (Krasny-Pacini & Evans, 2018). According to this methodology, the primary goal is to evaluate the effectiveness of the intervention implemented. To meet high-standard criteria, a minimum of three participants, each with at least three data points per variable of interest across different phases, is required (Kratochwill et al., 2010).

Based on study methodology, all five participants began the study simultaneously in the baseline phase, during which their performance levels were observed and documented. The intervention phase was then introduced in a staggered manner, with one participant at a time transitioning from the baseline to the intervention phase. This staggered introduction allowed researchers to attribute any observed changes in behavior or performance to the intervention, ensuring that these changes were not influenced by external factors (Krasny-Pacini & Evans, 2018; Zhan & Ottenbacher, 2001).

Accordingly, each participant acted as their own control, and the staggered introduction served as a replication of the intervention's effects across different individuals. This design strengthened the study's internal validity by facilitating clear cause-and-effect inferences (Zhan & Ottenbacher, 2001). The results demonstrated that the intervention, specifically in-phase bilateral exercises for the upper limbs, was the sole cause of improvement in the participants' conditions. While outcome measures remained unchanged for participants who were still in the baseline phase, they improved for those who had transitioned to the intervention phase (Figure 2.21).

To ensure the reliability of the results, especially in the case of dropouts, five participants were included in the study. Data collection involved recording several data points during the baseline phase, five data points during the intervention phase, and three data points during the follow-up phase. Demographic and baseline clinical characteristics of the participants were collected prior to the intervention and are summarized in Table 2.5. Also, individual MRI slices are presented in Appendix VII - Individual MRI slices.

Table 2.5. Participants' demographic and baseline clinical characteristics – multiple baseline design.

Participant	**A**	**B**	**C**	**D**	**E**
Age (years)	56	56	47	52	59
Sex	Female	Female	Female	Female	Female
Dominant hand	Right	Right	Right	Right	Right
Weight (kg)	83	57	50	66	55

Height (m)	1.68	1.58	1.55	1.68	1.60
BMI	29.4 (overweight)	22.8 (normal)	20.8 (normal)	23.4 (normal)	21.5 (normal)
HR[a] max (bpm)	164	164	173	168	161
Left upper limb length[b] (cm)	69	61	60	68	67
Right upper limb length[b] (cm)	69	61	60	68	67
EDSS	3	3.5	3.5	3	3.5
Disease duration (years)	9	7	3	9	8
Current clinical symptoms	- General weakness. - Minor imbalance during gait.	- General weakness. - Minor imbalance during gait. - Spasticity: grade one on both feet according to the Modified	- General weakness. - Minor imbalance during gait and standing. - Spasticity: grade one on both feet according to the	- General weakness. - Minor imbalance during gait.	- General weakness. - Minor imbalance during gait.

		Ashworth Scale	Modified Ashworth Scale		
MMSE (Folstein et al., 1975)	30 (no cognitive impairment)	30 (no cognitive impairment)	30 (no cognitive impairment)	30 (no cognitive impairment)	30 (no cognitive impairment)
MS related medication	Gilenya (Kappos et al., 2015; Zécri, 2016)	Gilenya (Kappos et al., 2015; Zécri, 2016)	Ocrelizumab (Syed, 2018)	Gilenya (Kappos et al., 2015; Zécri, 2016)	Aubagio (Bar-Or, 2014)
Occupation	Sedentary	Sedentary	Sedentary	Sedentary	Sedentary

Table 2.5: BMI; Body Mass Index, HR; Heart Rate, EDSS; Expanded Disability Status Scale, MMSE; Mini Mental State Examination, MS; Multiple Sclerosis. [a]; Heart Rate maximum was calculated from the equation 220-age (Tanaka et al., 2001b). [b]; Upper limbs length were measured in an anatomical position from C7 spinous process to the ulnar head (Livingston et al., 2010). All participants were right-handed, in the same decade of age and with similar body characteristics. Because upper limb length is a factor contributing to MEPs responses (Livingston et al., 2013), both upper limbs' length were measured for all participants, without a difference between both sides. EDSS score for all participants was between 3 and 3.5, which indicating moderate disability (Kurtzke, 1983). The MMSE and current clinical symptoms were in line with inclusion/exclusion criteria, so there was no specific impact related to the TMS and clinical measures. All participants were engaged in sedentary work (Thivel et al., 2018; Tremblay et al., 2017), which required low physical demands and no frequent moves; thus, it may not have an impact on the study's outcome measures.

Figure 2.21. Timeline and schematic representation of the multiple baseline study design.

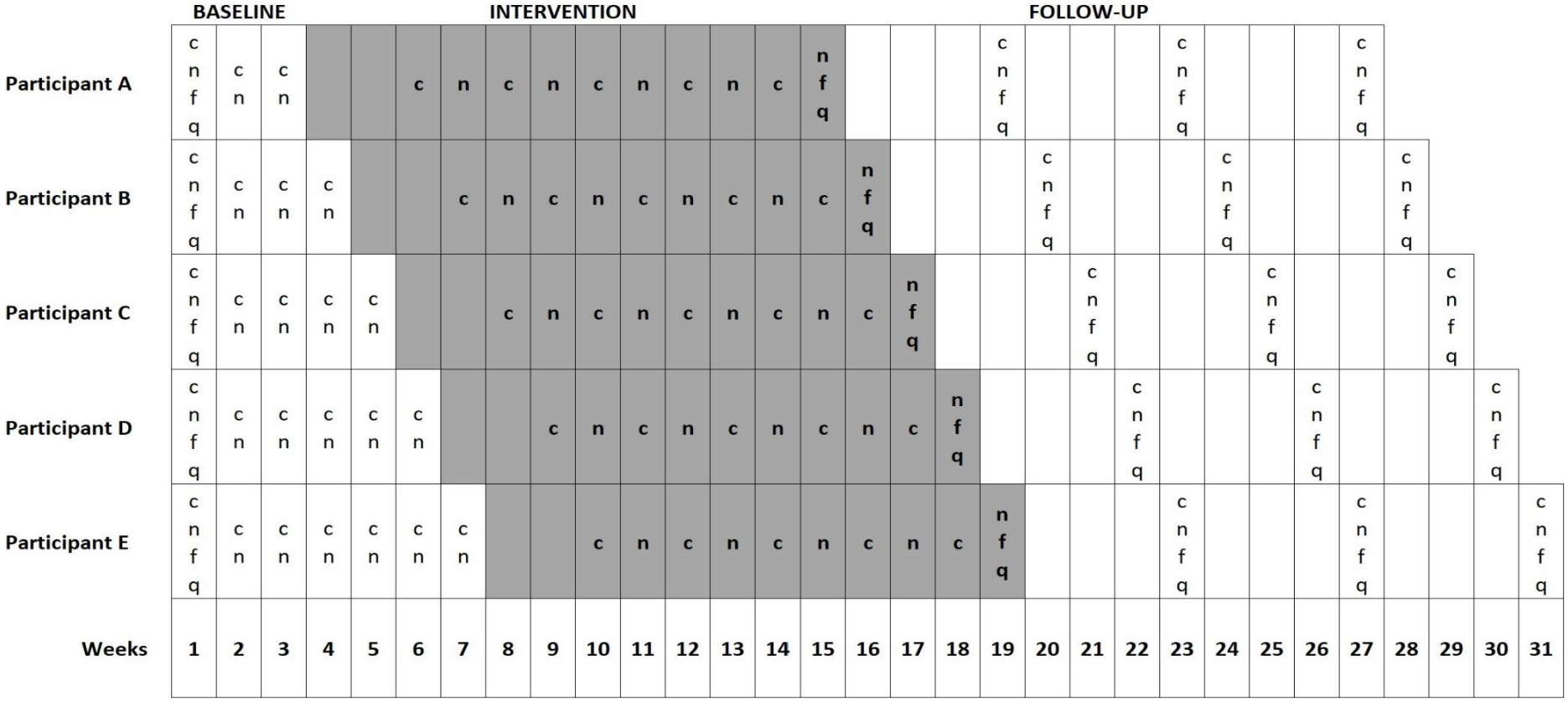


Figure 2.21: c; clinical assessment, n; neurophysiological assessment via TMS f; Modified Fatigue Impact Scale questionnaire, q; Medical Outcomes Study Questionnaire Short Form 36 Health Survey. Grey color represents the intervention phase. Each row (A–E) represents a different participant. Every cell represents a different week, so every procedure which is included (i.e., c, n, f, q) was performed during the corresponding week but in different days.

Baseline

As depicted in Figure 2.21, all patients began the baseline phase simultaneously. Each patient underwent a baseline phase of a different time duration (3 – 7 weeks), starting with three weeks for the first participant and gradually increased by one week for each participant. During the baseline phase, each participant was assessed on the Modified Fatigue Impact Scale (Fisk, Ritvo, et al., 1994) and Medical Outcomes Study Questionnaire Short Form 36 Health Survey (Lins & Carvalho, 2016) during the first week. The neurophysiological (i.e., CMCT, rMT, MEPs amplitude and latency) and clinical (i.e., gait, balance, strength, hand dexterity, cognitive functions) assessments were repeated after each baseline week for all participants.

Intervention

Immediately after the end of each baseline phase, the intervention phase began staggered across participants and time accordingly (Figure 2.21). The intervention protocol was based on the same protocol which was applied to the single case pilot study.

The intervention phase for each participant consisted of 12 consecutive weeks in which the protocol was performed three times per week, for 30 – 60 minutes each session, adapted to each participant's fatigue and fitness level. Each participant had to complete at least 27 (75%) out of 36 sessions in order for participant's data to be included in the analysis (Akbar et al., 2020). Every intervention session consisted of a five-minute warm-up (i.e., whole body range of motion exercises), followed by the main sport activities and fitness exercise protocol as described above, and a cool down for five minutes (i.e., passive stretching exercises of the muscle groups which are involved in the main part).

Additionally, starting from the third intervention week, five neurophysiological and five clinical assessments (i.e., once a week) were performed, to collect five data points for every participant across the intervention phase. Moreover, each participant was asked to complete the Modified Fatigue Impact Scale and Medical Outcomes Study Questionnaire Short Form 36 Health Survey (see secondary measures), once, at the end of the intervention phase (Akbar et al., 2020) (Figure 2.21).

The exercise protocol lasted 12 consecutive weeks and contained three sets of 12 exercises within each set, with two minutes rest between the sets. All details regarding

the exercise protocol are presented in Table 2.6 and also in Appendix IV - Examples of the exercise protocol**.**

Table 2.6. Overview of the exercise protocol – multiple baseline design.

	Type of exercise	Repetitions	Body position	Difficulty level
1	Basketball chest pass	20-30	Standing	Distance of the pass
2	PNF 1st diagonal FP	20-30	Standing	Elastic band
3	Flexion of all fingers	20-30	Sitting	Hand training net
4	Adductors squeeze	20-30	Supine lying	Pilates ring
5	Basketball shoulder pass	20-30	Standing	Distance of the pass
6	PNF 1st diagonal EP	20-30	Standing	Elastic band
7	Extension of all fingers	20-30	Sitting	Hand training net
8	Hips Abduction	20-30	Supine lying	Pilates ring
9	Volleyball overhead pass	20-30	Standing	Distance of the pass
10	PNF 2nd diagonal FP	20-30	Standing	Elastic band
11	PNF 2nd diagonal EP	20-30	Standing	Elastic band
12	Squat	20-30	Standing	Balance pads

Table 2.6: PNF; Proprioceptive Neuromuscular Facilitation, FP; Flexion Pattern, EP; Extension Pattern. Each session included three sets of nine different exercises which targeted large muscle groups of the upper limbs (i.e., 1–3, 5–7, 9–11) and three exercises which targeted large muscle groups of the lower limbs (i.e., 4, 8, 12). Overall, for all participants the range of repetitions was 20 – 30 according to individuals' fitness level. The difficulty level for exercises 4 and 8 was maintained by changing the resistance of the Pilates ring, whereas for exercise 12 the difficulty level was sustained by changing the base of support (e.g., balance pads, Bosu ball). The difficulty level for the sport activities (i.e., 1, 5, 9) was maintained by changing the distance of the passes. All PNF exercises (i.e., 2, 6, 10, 11) were performed against the resistance of elastic bands (different resistance accordingly), which it was attached by a stable point.

For the duration of the intervention implementation period, there was a continuous monitoring and record of the participants' performance. Individual performance data are presented in Table 2.7.

Table 2.7. Individual performance during the exercise protocol.

Participant	**A**	**B**	**C**	**D**	**E**
Sessions Completed	32/36	31/36	32/36	32/36	34/36
Number of Repetitions	821	824	945	956	773
HR (bpm)	93	108	102	94	101
%HR maximum	57	66	62	56	63
RPE	4	4	5	5	3
Body Temperature(℃)	36.1	36.3	35.3	35.7	36.2
Resistance Level	Medium	Light	Medium	Medium	Medium

Table 2.7: HR; Heart Rate RPE; Rating of Perceived Exertion. Individual mean values from the total completed number of sessions are presented in Table 2.7. All participants completed more than 75% (group mean; 89%, 32/36 sessions) of the total intervention sessions, which was set as the minimum accepted percentage of completed sessions per participant. Furthermore, all participants exceeded the recommended number (i.e., 300) of repetitions required in a session to induce neuroplastic effects (Catherine et al., 2016). Additionally, all of them completed the exercise protocol following the recommended exercise features regarding HR, RPE, body temperature and resistance level (Foster et al., 2001; Kalb et al., 2020). However, the percentage of the maximum HR indicated that none of them exceeded the aerobic level of exercise (i.e., below 70% of each participant's maximum HR) (Skinner & McLellan, 1980) during the sessions. In summary, for all participants the range of the exercise HR was 56% – 66% of the individual maximum HR, the range of RPE (Foster et al., 2001). was 3 – 5 (on a 10-point scale) and the range of body temperature during exercise was 35.3℃ – 36.3℃.

Since the main aim of this study was to induce neuroplasticity as an effect of the specific type of movement (i.e., in-phase bilateral movement of the upper limbs) rather than as a

training effect (Diechmann et al., 2021; Hortobágyi et al., 2021; Learmonth & Motl, 2021; Pickersgill et al., 2022) participants' individual performance was maintained with the HR zone for aerobic exercise (i.e., below 70% of each participant's maximum HR (Skinner & McLellan, 1980)).

Considering that the individual HR should be within the range of 50 – 70% of the individual maximum HR, the progression and difficulty level were gradually enhanced. The progression was managed by increasing the distance of the passes and the resistance of the elastic bands according to each exercise. This approach ensured that the exercises remained challenging yet achievable, maximizing the benefits for participants without risking overexertion or exceeding their individual aerobic capacity.

As it was described in the single-case pilot study, aerobic exercise at 70% of an individual's maximum HR is considered moderate to vigorous intensity and is particularly effective for promoting neuroplasticity (Cotman et al., 2007; Maass et al., 2015; Skinner & McLellan, 1980). Additionally, pwMS are particularly sensitive to changes in body and environmental temperature, due to Uhthoff's phenomenon (Frohman et al., 2013). To address this, individual HR and body temperature were continuously monitored for all participants.

To maintain a consistent HR and body temperature, a pulse oximeter (ChoiceMMed OxyWatch C29, Bristol, United States) was used for HR screening, and a forehead thermometer was employed to monitor body temperature at the end of each set during all sessions. Room temperature was controlled at 24°C, ensuring that all participants exercised under the same conditions.

Follow-up

As depicted in Figure 2.21, every participant underwent three follow-up assessments in total, after finishing the training protocol, so to explore possible long-lasting effects. Each follow-up assessment included both neurophysiological and clinical measures. The first follow-up assessment was performed at the end of the fourth post-intervention week, the second one at the end of the eighth post-intervention week and the last follow-up assessment at the end of the 12^{th} post-intervention week.

Possible Threats

During the study implementation, different threats could be present and could affect internal validity of the study (Kratochwill et al., 2010). Attrition was one threat (Kratochwill et al., 2010), which might have an impact on the experimental conditions in the case of less than three participants and less than three data points in each phase were presented (Krasny-Pacini & Evans, 2018).

Given that, a specific methodology was employed, which included five participants and at least three assessments points per participant, throughout all phases (i.e., baseline, intervention, follow-up) so to avoid attrition (Figure 2.21). Additionally, according to the exercise protocol, participants had to complete at least 75% of the total intervention sessions, therefore this did not affect the implementation of this study in case of an absence during the intervention phase.

History is another possible threat (Kratochwill et al., 2010). Because it might present a limited ability to explore what other events would probably influenced the outcome measures, each participant was asked to have a written calendar of their daily routine (e.g., any other physical activity, occupational and pharmaceutical changes) throughout the study duration. Also, by using the specific study design (i.e., single-case multiple baseline design) the present of this thread was eliminated, because an advantage to monitor and examine individual behavior through the repetitive data collection during baseline and intervention phases were able. Moreover, to ensure that participants did not make other outcome-related changes in their daily life, they were advised prior to the study implementation to continue their usual prescribed medication throughout the study duration. However, none of the participants made any changes to their usually prescribed medication upon physician recommendation.

2.3.1 Results - Primary outcome measure

Following study's sampling plan, a total of five participants were recruited following the inclusion criteria. All participants completed all assessments (Figure 2.21) and the exercise protocol without complaints or side effects. Participant E missed one assessment data point at baseline and another assessment during the intervention phase. Despite these omissions, Participant E' data were retained and included in the overall analysis.

Central Motor Conduction Time

A decrease in CMCT signifies an improvement. The results of the CMCT assessments during all time points are depicted in Figure 2.22 and they are also presented in Appendix VIII_Multiple Baseline – Results; Table 1. Moreover, individual raw data are presented in Appendix IX - Individual EMG raw data.

Figure 2.22. Visual representation of the Central Motor Conduction Time during baseline, intervention and follow-up phases – multiple baseline study.

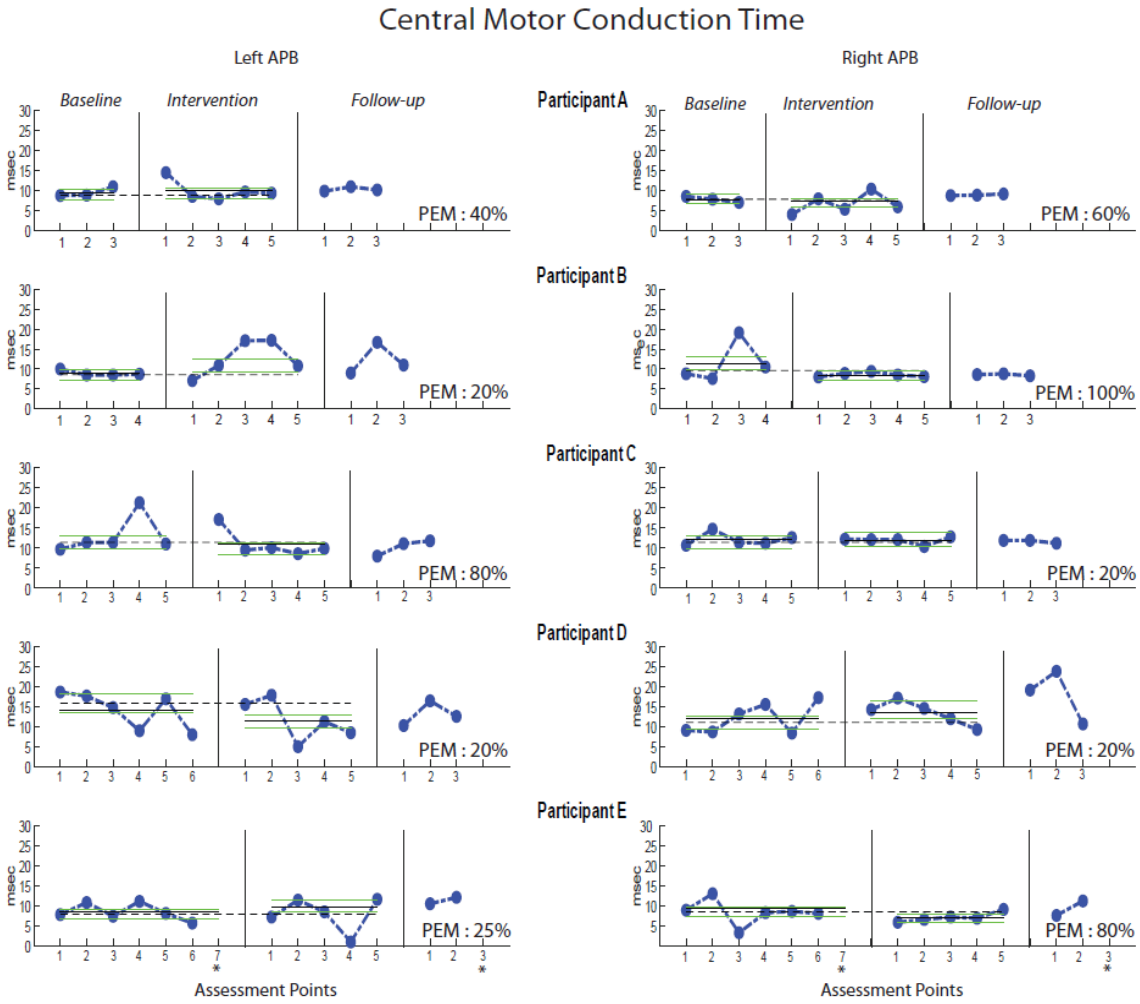


Figure 2.22: APB; Abductor Pollicis Brevis, PEM; Percentage Exceeding the Median. (*) denotes the missed assessment points, which Participant E couldn't perform (i.e., last TMS assessment of baseline and follow-up phases bilateral). Data of each participant are presented regarding left and right upper limb, in terms of left and right APB. The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the values of the central motor conduction time are presented. Central motor conduction time, is measured by means of milliseconds (msec). The vertical lines between the data points indicate the three study phases (i.e., baseline, intervention, follow-up). The area between the green lines, refers to the acceptable range regarding the stability

criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases only. The black horizontal lines represent the within-phase mean. Although, the mean lines for all baseline phases may not be clearly visible as they are superimposed with the PEM lines.

Within-phase visual analysis: Data for both left and right upper limbs were analyzed in terms of variability (i.e., the spread of data points within a phase that was indicated from the two-standard deviation band, which is the mean of a phase and adding and subtracting two standard deviations from it (Krasny-Pacini & Evans, 2018), stability (Lobo et al., 2017) and trend. Low variability (i.e., 0-20%), was observed for all participants except for Participant E who showed high variability on both the baseline (50%) and the intervention (40%) phase for the left upper limb, whereas high variability (40%) was observed for the right upper limb during the baseline phase, but not during the intervention phase (0%).

Stability of the data (>80% (Lobo et al., 2017)) was observed for Participants A (baseline: 100%, intervention: 80%) and C (baseline: 80%, intervention: 80%) for the left upper limb. Participants B (intervention: 100%), C (baseline: 80%, intervention: 100%) and E (intervention: 80%) were the only ones who met the stability criterion for the right upper limb.

In terms of the trends of the CMCT, during the baseline phase all directions must be stable, whereas during the intervention phase downward or stable (given that there is a change in level between phases) directions signify an improvement. During the baseline phase, three out of five participants (B, D, E) showed downward trends, whereas two out of five (A, C) showed upward trends for the left upper limb. During the intervention phase, Participants A, B, C and D showed downward trends, while Participant E showed a stable trend for the left upper limb. On the other hand, during the baseline phase only Participant B showed stable trend, while Participants A and C showed downward trends and Participants D and E showed upward trends, for the right upper limb. However, during the intervention phase three out of five participants (A, C, D) showed downward trends, while Participants B and E showed downward and stable trends respectively for the right upper limb. As a conclusion, none of the participants met all the within-phase visual analysis' criteria, for either upper limb.

Between-phases visual analysis: Data from both baseline and intervention phases were included in the between-phases analysis. The criteria which were used for the visual analysis, were level (i.e., change of mean values between phases), immediacy (i.e., change in level between the last three data points of the baseline phase and the first three data points of the intervention phase) and the PEM (MA, 2006). Reduction (i.e., improvement) of mean values was observed for Participants C (baseline: 13 ms, intervention: 11 ms) and D (baseline: 14.2 ms, intervention: 11.6 ms), for the left upper limb, while participants A (baseline: 7.7 ms, intervention: 7.3 ms), B (baseline: 11.4 ms, intervention: 8.5 ms), C (baseline: 12.1 ms, intervention: 11.9 ms) and E (baseline: 9.3 ms, intervention: 7.1 ms), showed reduction of mean values for the right upper limb. Therefore, participants C and D showed a difference in level for the left upper limb, though Participants A, B, C and E showed a difference in level and immediate effect for the right upper limb. The PEM (MA, 2006) indicates an effect when 70% of data of the intervention phase exceed the median of the baseline phase. According to our results, only Participant C (PEM = 80%) showed an effect for the left upper limb of, whereas Participants B (PEM = 100%) and E (PEM = 80%) showed an effect for the right upper limb. In summary, none of the participants met all visual analysis' criteria (Lobo et al., 2017); therefore, we did not proceed with statistical analysis of those data, in line with our pre-registered analysis plan.

2.3.2 Results - Secondary outcome measures

Following our sampling plan, a total of five participants were recruited following the inclusion criteria. All participants completed all assessments (Figure 2.21) and the exercise protocol without complaints or side effects. Participant E missed one assessment data point at baseline and another assessment during the intervention phase. Despite these omissions, Participant E' data were retained and included in the overall analysis.

Motor Evoked Potentials Amplitude

Throughout the within and between-phases visual analysis, no evidence of improvement related to MEPs amplitude was observed for any of the participants. The results of the MEPs amplitude assessments during all time points are depicted in Figure 2.23 and presented in Appendix VIII_Multiple Baseline - Results; Table 2. Moreover, individual raw data are presented in Appendix IX - Individual EMG raw data.

Figure 2.23. Visual representation of the Motor Evoked Potentials Amplitude during baseline, intervention and follow-up phases – multiple baseline study.

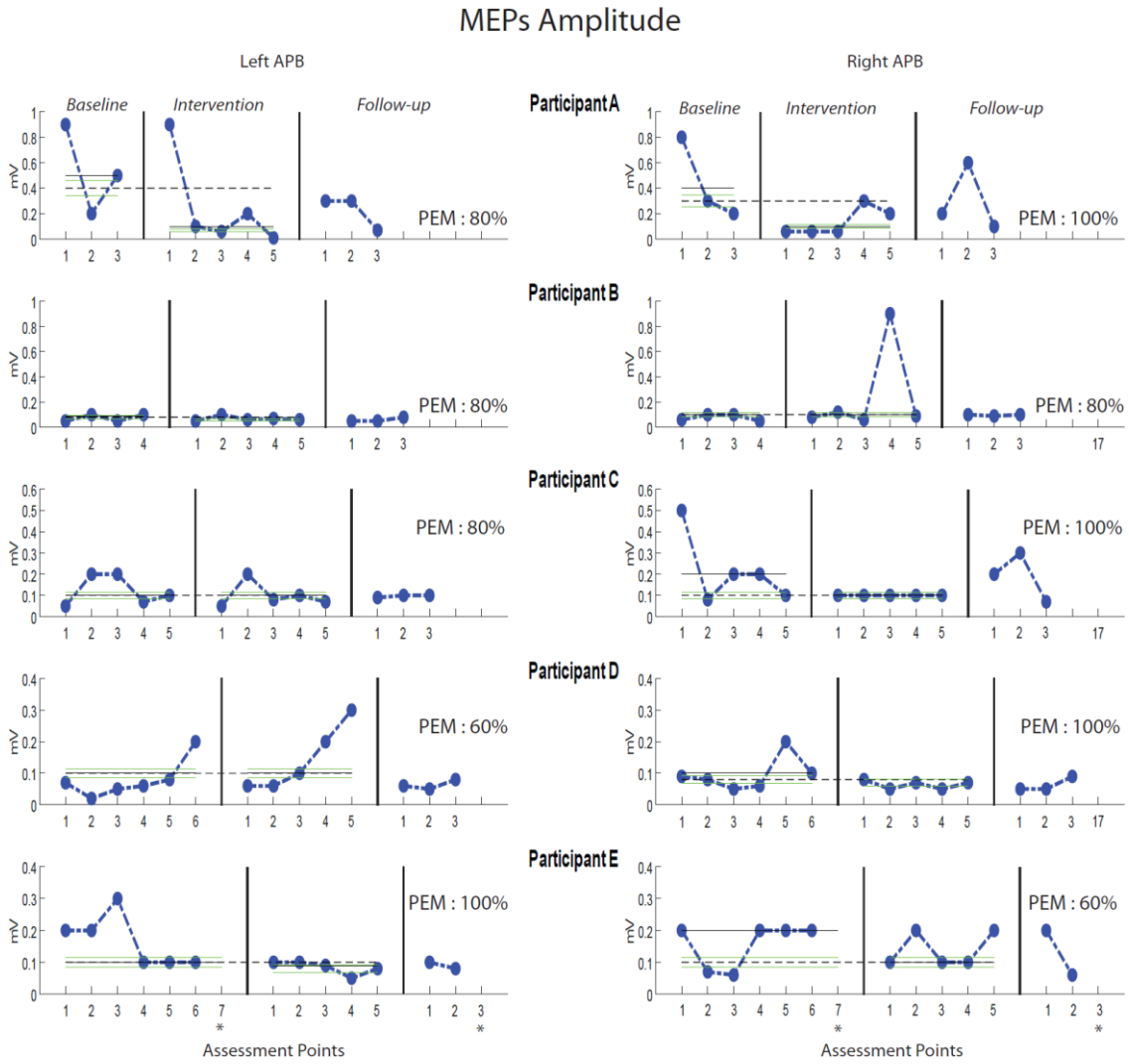


Figure 2.22: MEPs; Motor Evoked Potentials, APB; Abductor Pollicis Brevis, PEM; Percentage Exceeding the Median. Data of each participant are presented regarding left and right upper limb, in terms of left and right APB. The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the values of the MEPs peak-to-peak amplitude are presented. MEPs peak-to-peak amplitude, is measured by means of millivolts (mV). The values on the y axis are different between participants indicating intersubject variability. The vertical lines between the data points indicate the three study phases (i.e., baseline, intervention, follow-up). The area between the green lines, refers to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017)). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases only. The black horizontal lines represent the within-phase mean. Although, the mean lines for all

baseline phases may not be clearly visible as they are superimposed with the PEM lines. (*) denotes the missed assessment points, which Participant E couldn't perform (i.e., last TMS assessment of baseline and follow-up phases bilateral).

An increase in MEPs amplitude signifies an improvement. Only Participant D (baseline mean = 0.08mV, intervention mean = 0.1mV) showed an improvement for the left upper limb, whereas Participants A (baseline mean = 0.47mV, intervention mean = 0.1mV) and B (baseline mean = 0.09mV, intervention mean = 0.1mV) showed an improvement for the right upper limb. Participants A (left upper limb), B (left upper limb) and D (right upper limb) showed an improvement of MEPs amplitude, but low data stability was observed on both baseline and intervention phases for these three participants (A: baseline = 33%, intervention = 40%; B: baseline = 0%, intervention = 40%; C: baseline = 33%, intervention = 40%).

Motor Evoked Potentials Latency

Throughout the within and between-phases visual analysis, no evidence of improvement related to MEPs latency was observed for any of the participants. The results of the MEPs latency assessments during all time points are depicted in Figure 2.24 and they are also presented in Appendix VIII_Multiple Baseline - Results; Table 3. Moreover, individual raw data are presented in Appendix IX - Individual EMG raw data.

Figure 2.24. Visual representation of the Motor Evoked Potentials Latency during baseline, intervention and follow-up phases – multiple baseline study.

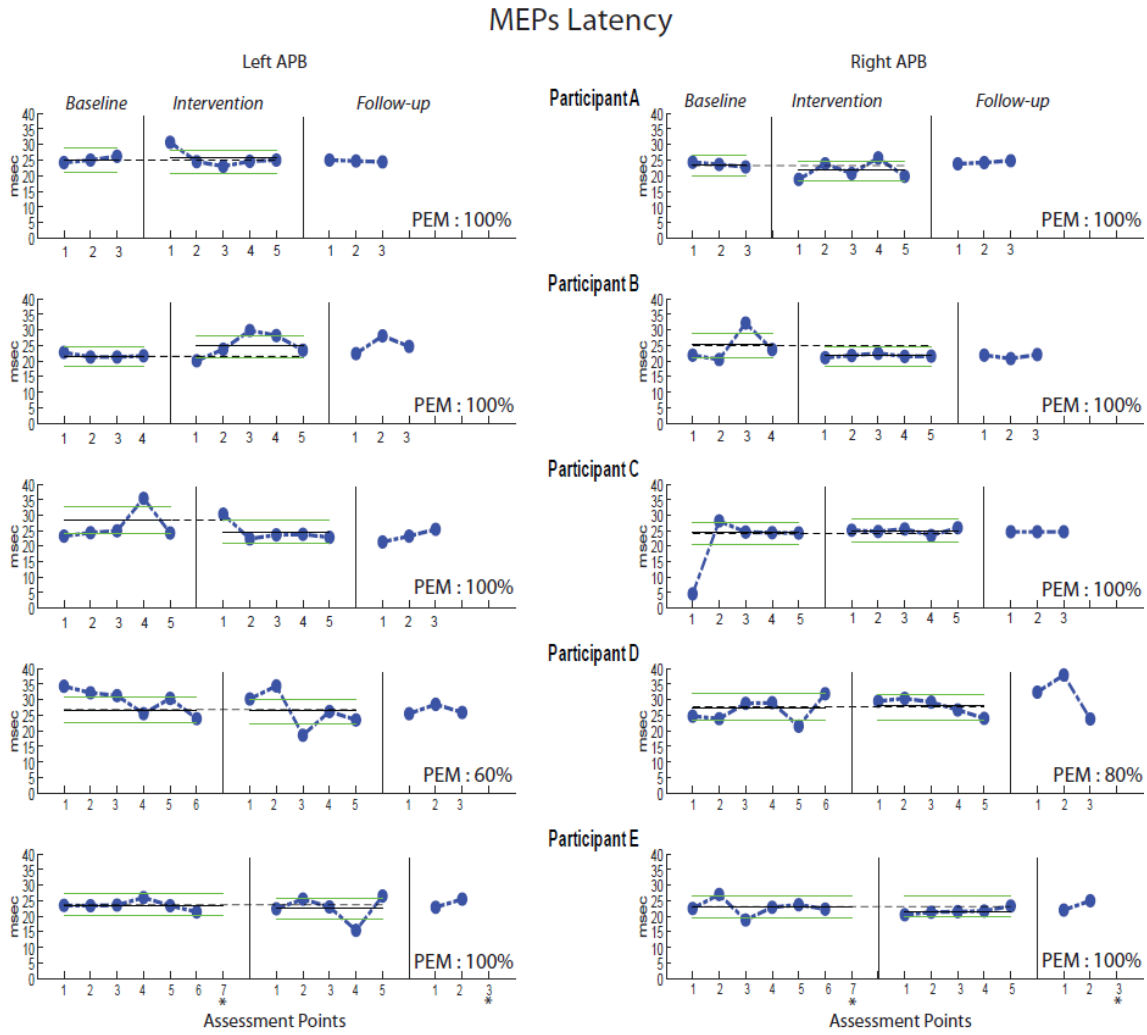


Figure 2.24: MEPs; Motor Evoked Potentials, APB; Abductor Pollicis Brevis, PEM; Percentage Exceeding the Median. Data of each participant are presented regarding left and right upper limb, in terms of left and right APB. The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the values of the MEPs latency are presented. MEPs latency, is measured by means of milliseconds (msec). The vertical lines between the data points indicate the three study phases (i.e., baseline, intervention, follow-up). The area between the green lines, refers to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases only. The black horizontal lines represent the within-phase mean. Although, the mean lines for all baseline phases may not be clearly visible as they are superimposed with the PEM lines. (*) denotes the missed assessment

points, which Participant E couldn't perform (i.e., last TMS assessment of baseline and follow-up phases bilateral).

Reduction in MEPs latency signifies an improvement. Three out of five participants (C: baseline mean = 26.4 ms, intervention mean = 24.5 ms; D: baseline mean = 29.6 ms, intervention mean = 26.6 ms; E: baseline mean = 23.5 ms, intervention mean = 22.6 ms) showed an improvement on the left upper limb measures, whereas four out of five participants (A: baseline mean = 23.6ms, intervention mean = 21.7ms; B: baseline mean = 24.6ms, intervention mean = 21.7ms; C: baseline mean = 25.1ms, intervention mean = 24.9ms; E: baseline mean = 22.9ms, intervention mean = 21.6ms) showed an improvement on the right upper limb measures.

Even though an improvement was observed in the mean values, low data stability and unexpected trend directions were detected. To meet the stability criterion, the data on any of the phases needs to be more than 80% and to meet the trend criterion, the expected directions needs to be stable during the baseline and downward or stable (given that there is a change in level between phases) during the intervention phase (Lobo et al., 2017). Participant C showed an upward trend during baseline, Participant D showed a downward trend and low data stability (66%) during baseline and Participant E showed an upward trend and low data stability (60%) during the intervention, for the left upper limb. Participants A and C showed a downward trend during baseline, Participant B showed an upward trend during baseline and Participant E showed low data stability (66%) during baseline, for the right upper limb. Therefore, none of the participants could be included for statistical analysis for the MEPs amplitude and latency.

Resting Motor Threshold

A decrease in rMT signifies an improvement. The repeated assessments of the rMT across all assessment time points are depicted in Figure 2.25 and they are also presented in Appendix VIII_Multiple Baseline - Results; Table 4.

Figure 2.25. Visual representation of the Resting Motor Threshold during baseline, intervention and follow-up phases – multiple baseline study.

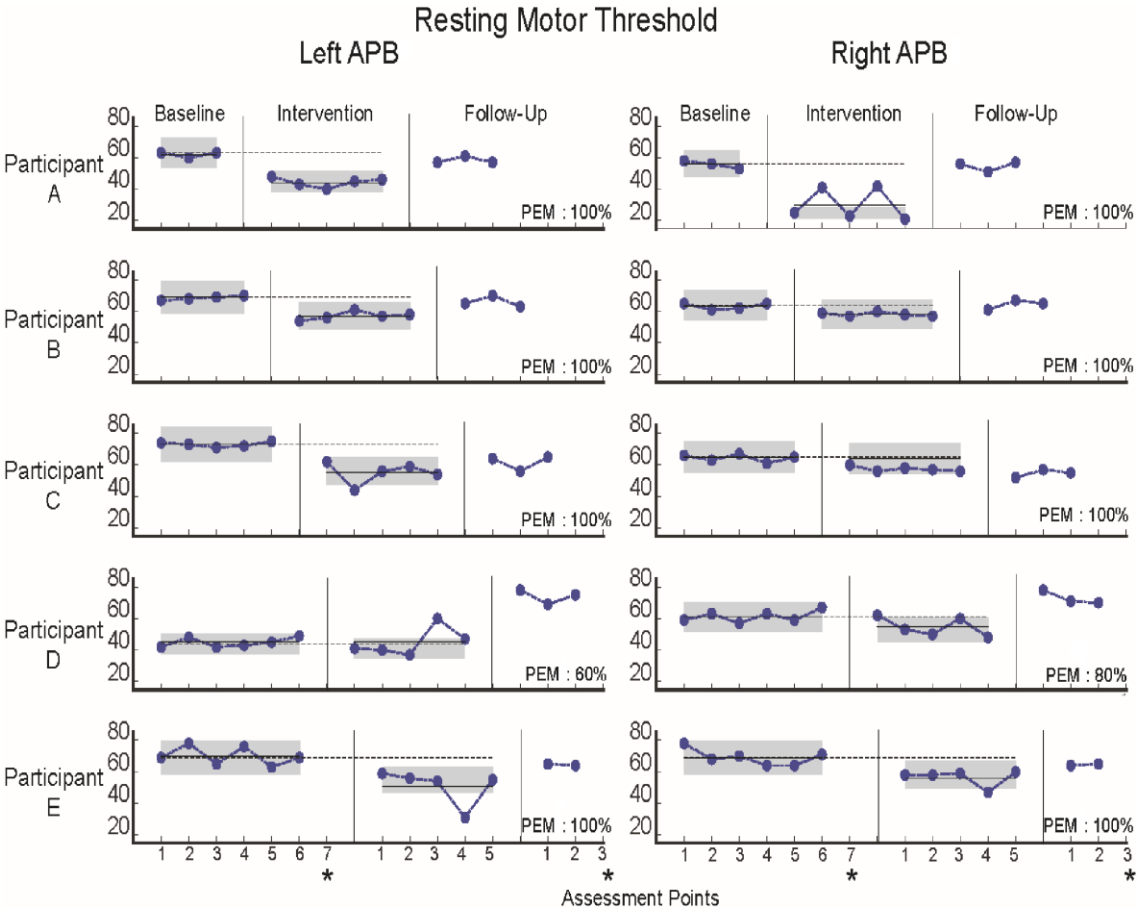


Figure 2.25: APB; Abductor Pollicis Brevis, PEM; Percentage Exceeding the Median. Data of each participant are presented regarding left and right upper limb, in terms of left and right APB. The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the values of the rMT are presented. Resting motor threshold, is measured by means of the percentage of the Maximum Stimulator Output (%MSO). The vertical lines between the data points indicate the three study phases (i.e., baseline, intervention, follow-up). The grey area around the data points, refers to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Krasny-Pacini & Evans, 2018; Lobo et al., 2017)). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases only. The black horizontal lines represent the within-phase mean. Although, the mean lines for all baseline phases may not be clearly visible as they are superimposed with the PEM lines. While on the intervention phase the mean lines are well seen, thus indicating the effect of the intervention (except that of Participant D; left APB). Also, some data points during the intervention phase may be detected to be outside of the data stability range (Participant C: second intervention point for the left APB and Participant E: fourth intervention point bilaterally), yet the

percentage of the within-phase stability for them is greater than the accepted 80% (Krasny-Pacini & Evans, 2018; Lobo et al., 2017). (*) denotes the missed assessment points, which Participant E couldn't perform (i.e., last TMS assessment of baseline and follow-up phases bilateral). During the follow-up phase all data showed a tendency to return towards the baseline level.

Within-phase visual analysis: Data were analyzed in terms of variability (Krasny-Pacini & Evans, 2018), stability (Lobo et al., 2017) and trend for both left and right upper limbs. All participants showed no variability during both baseline and intervention phases, for the left upper limb. The criterion of data stability and the expected trend direction (i.e., baseline: stable trend, intervention: downward or stable trend given that there is a decreased level compared to baseline phase, follow-up: upward or stable trend given that there is a decreased level compared to baseline phase) was met from Participants A (baseline: stability = 100%, stable trend; intervention: stability = 100%, stable trend), B (baseline: stability = 100%, stable trend; intervention: stability = 100%, stable trend), C (baseline: stability = 100%, stable trend; intervention: stability = 100%, stable trend) and E (baseline: stability = 100%, stable trend; intervention: stability = 80%, downward trend), for the left upper limb. Although, Participant D showed data stability on both baseline (100%) and intervention (80%) phases, upward trends (i.e., unexpected direction) presented on both baseline and intervention phases, for the left upper limb. Therefore, all participants met all criteria from the within-phase visual analysis for the left upper limb, except from Participant D who showed unexpected trends directions.

No variability for all participants was observed on both baseline and intervention phases for the right upper limb. However, only Participants B (baseline: stability = 100%, stable trend; intervention: stability = 80%, downward trend), C (baseline: stability = 100%, stable trend; intervention: stability = 100%, downward trend) and E (baseline: stability = 100%, stable trend; intervention: stability = 80%, downward trend) met the criteria of data stability and trend, for the right upper limb. Although Participant A showed data stability during the baseline phase (100%), low data stability (60%) was observed during the intervention phase for the right upper limb. Also, Participant D showed data stability on both baseline (100%) and intervention (80%) phases, but an upward trend (i.e., unexpected direction) was observed during the baseline phase, for the right upper limb. Therefore, all participants met all criteria from the within-phase visual analysis for the right upper limb, except from Participants A who showed low data stability during the intervention phase and from Participant D, who showed an unexpected trend direction

(i.e., upward) during the baseline phase. Consequently, Participants A, B, C and E met all the within-phase criteria for the left upper limb, whereas Participants B, C and E met all criteria for the right upper limb.

Between-phases visual analysis: rMT data from both the baseline and the intervention phase were included in the between-phases analysis. The criteria used for the data analysis included the proportion of data overlap, level, immediacy and the PEM (MA, 2006). No data overlapping was observed for any participant, except for Participant D, who showed 40% of data overlapping for the left upper limb and 60% overlapping for the right upper limb. As shown in Figure 2.25, Participants A, B, C and E showed reduction of mean values for both left and right upper limbs, while Participant D showed reduction of mean value only for the right upper limb. All participants showed immediacy of the effect for both left and right upper limb, except for Participant D for the left upper limb. Moreover, the PEM data indicated that Participants A, B, C and E showed high effectiveness (100%) (MA, 2006) for both left and right upper limb. Participant D showed a moderate effect (PEM = 80%) only for the right upper limb, while no effectiveness (PEM = 40%) was found for the left upper limb. Participant D was the only one who did not satisfy the between-phases criteria.

In summary, throughout the within and between phases visual analyses, all criteria were met by Participants A, B, C and E for the left upper limb and by Participants B, C and E for the right upper limb.

Following our analysis plan, only the data from the participants who met the criteria from the visual analysis were included in statistical analysis (Kratochwill et al., 2010). As was indicated from the NAP index, Participant A (NAP = 1, $p < 0.05$) and Participants B, C and E (NAP = 1, $p < 0.01$) showed significant improvement of the rMT, for the left upper limb. Moreover, Participants B, C and E (NAP = 1, $p < 0.01$) showed significant improvement, for the right upper limb. The results of Participant A were not significant following a Bonferroni correction of 0.05/2, since we tested the same value for both upper limbs.

In general, four out of five participants (A, B, C, E) showed an improvement on the left upper limb measures, whereas three out of five participants (B, C, E) showed an improvement on the right upper limb measures. During the baseline phase, higher rMT for the left upper limb was observed (group mean = 64% MSO), compared to the right

upper limb (group mean = 62% MSO). During the intervention phase we found lower rMT for the left upper limb (group mean = 50% MSO), compared to the right upper limb (group mean = 52% MSO).

For the follow-up phase, a descriptive analysis was performed for all participants, indicating increase of the rMT for both left and right upper limbs. As was indicated from both left and right upper limbs mean values (left upper limb: group mean = 65% MSO; right upper limb: group mean = 62% MSO), all participants showed a trend to return in baseline values.

Clinical Assessment

The analysis indicated an improvement on all clinical assessments (i.e., Mini-Balance Evaluation Systems Test, Six Spot Step test, Action Research Arm Test, Hand Held Dynamometer, Symbol Digit Modalities Test, Modified Fatigue Impact Scale, Medical Outcomes Study Questionnaire Short Form 36 Health Survey) and a high level of agreement (percentage of agreement = 1) in three out of five clinical assessments (i.e., Mini-Balance Evaluation Systems Test, Six Spot Step test, Action Research Arm Test), across all study phases. The details and results of all phases are depicted in Figures 10.26 – 10.32 and the are also presented in Appendix VIII - Multiple Baseline – Results; Tables 5 – 9. All criteria of the visual analysis (Lobo et al., 2017) were satisfied for both within and between-phases analysis for all the participants, for both left and right upper limbs. Therefore, all participants and all clinical outcome measures were included in the statistical analysis.

Within-phase visual analysis: Data for both left and right upper limbs were analyzed in terms of stability and trend (Lobo et al., 2017). All participants showed no variability (Krasny-Pacini & Evans, 2018), high data stability (100%) (Lobo et al., 2017) and expected trends directions (baseline: stable trend; intervention: downward or stable trend given that there is a decreased level between phases, Follow-up: upward or stable trend given that there is a decreased level compared to baseline phase).

Between-phases visual analysis: Data form baseline and intervention phases were included in the between-phases analysis. The criteria used for the data analysis included level, proportion of data overlap, immediacy and the PEM (MA, 2006). All participants showed a change (i.e., improvement) in level, with no data overlapping between phases

for both left and right upper limbs. Also, all participants showed an immediate effect and a high level of effectiveness (PEM = 100%) (MA, 2006), for both left and right upper limbs. However, during the follow-up phase, all participants showed a minor reduction (i.e., decrease of the values) of the individual performance compared to the intervention phase, as was indicated throughout the individual data visual description.

Since all participants met all visual analysis criteria, they were all included in the statistical analysis, for all clinical outcome measure. The NAP index was used, which indicated significant results for all participants (NAP = 1, $p < 0.05$). The results for all clinical measures are depicted in Figures 2.26 – 2.32 and the are also presented in Appendix VIII_Multiple Baseline - Results; Tables 5 – 9. Although the results indicated nominal statistical significance, these would not survive a Bonferroni correction, if corrected for the total number of clinical tests performed. Nevertheless, this is an important observation in our pilot study that warrants further investigation.

Figure 2.26. Visual representation of the Mini Balance Evaluation Test during baseline, intervention and follow-up phases – multiple baseline study.

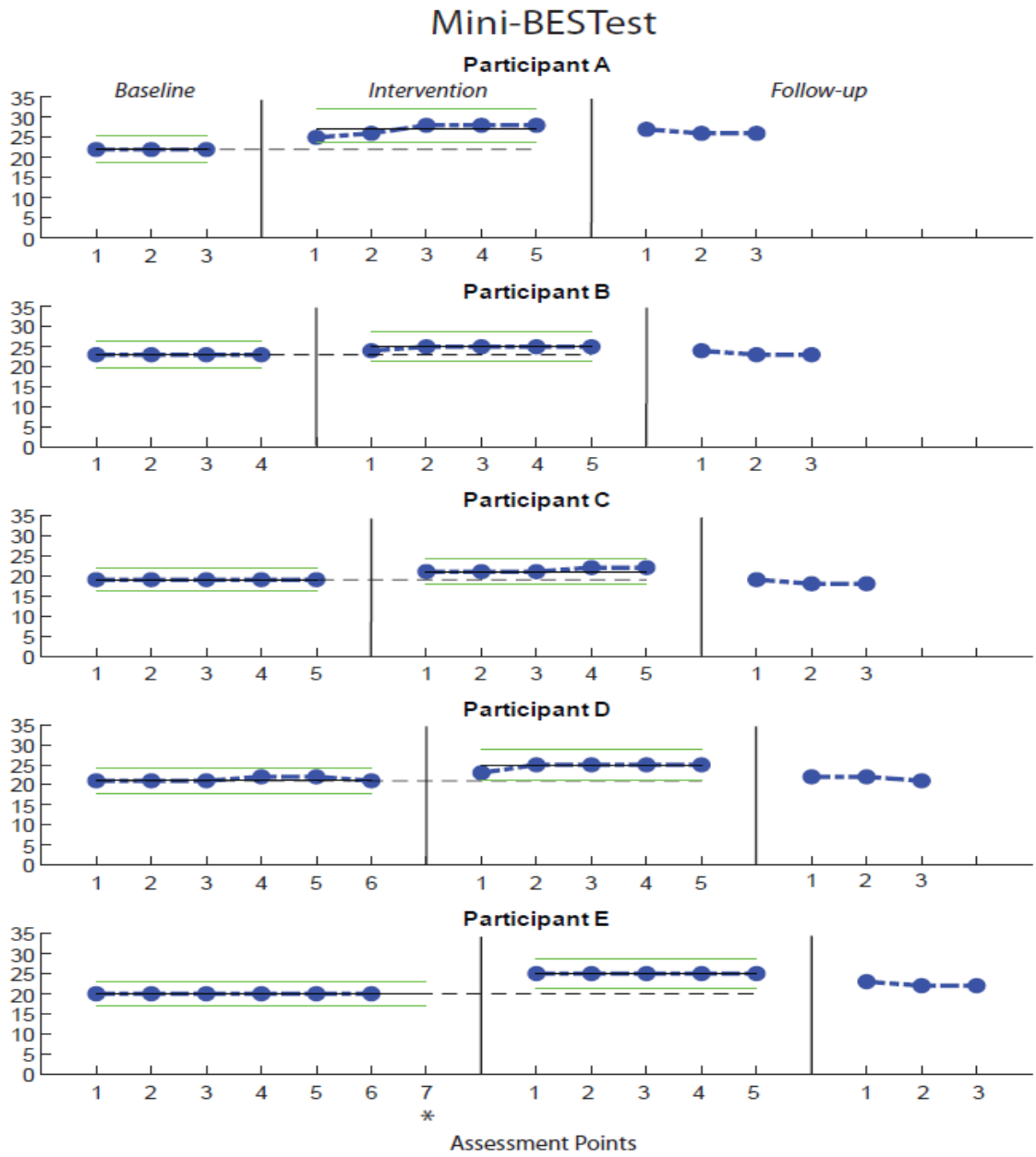


Figure 2.26: The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the scores of the test are presented. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases only. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Figure 2.27. Visual representation of the Six Spot Step Test during baseline, intervention and follow-up phases – multiple baseline study.

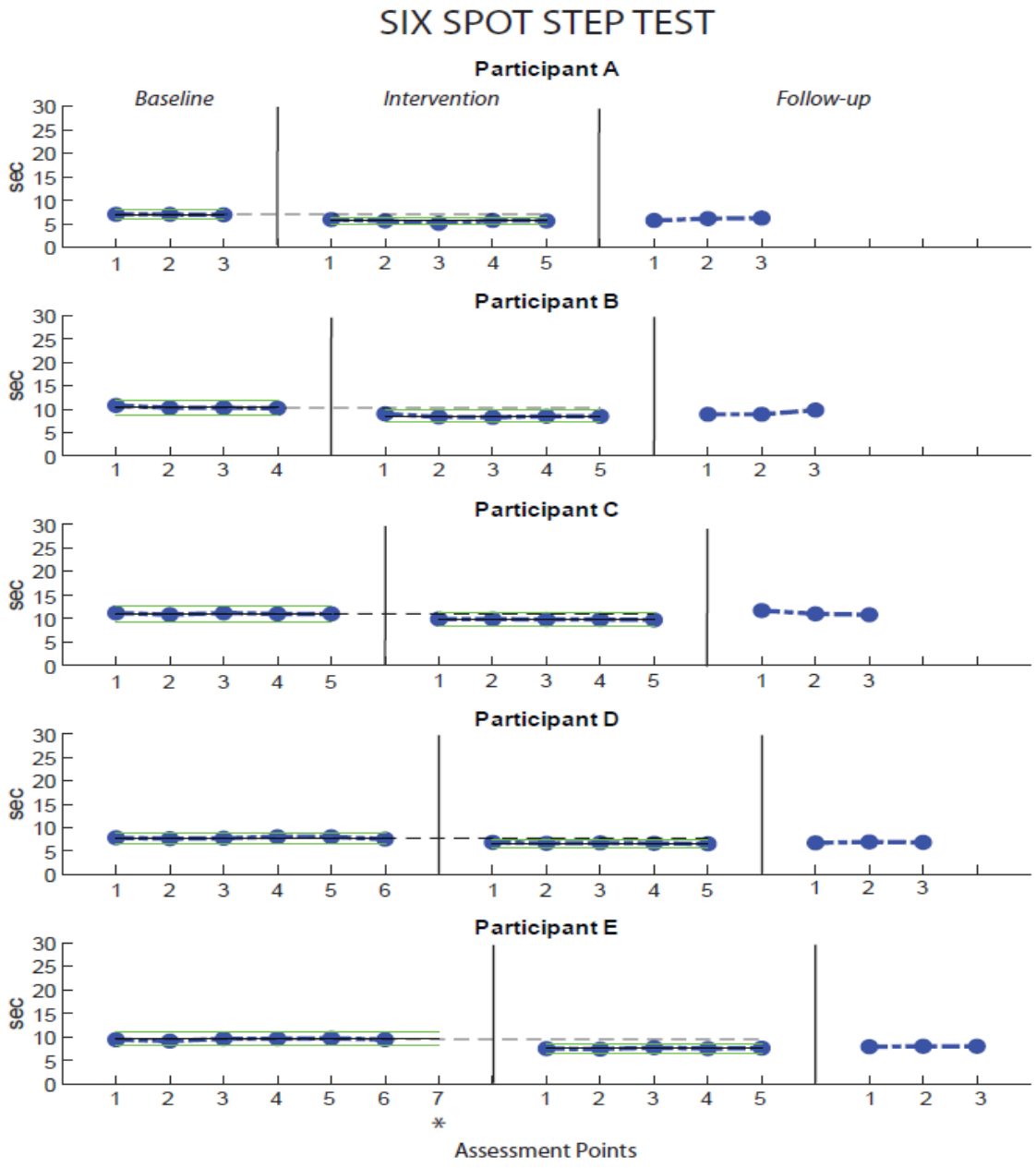


Figure 2.27: The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the scores (sec) of the test are presented. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases only. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Figure 2.28. Visual representation of the Symbol Digit Modalities Test during baseline, intervention and follow-up phases – multiple baseline study.

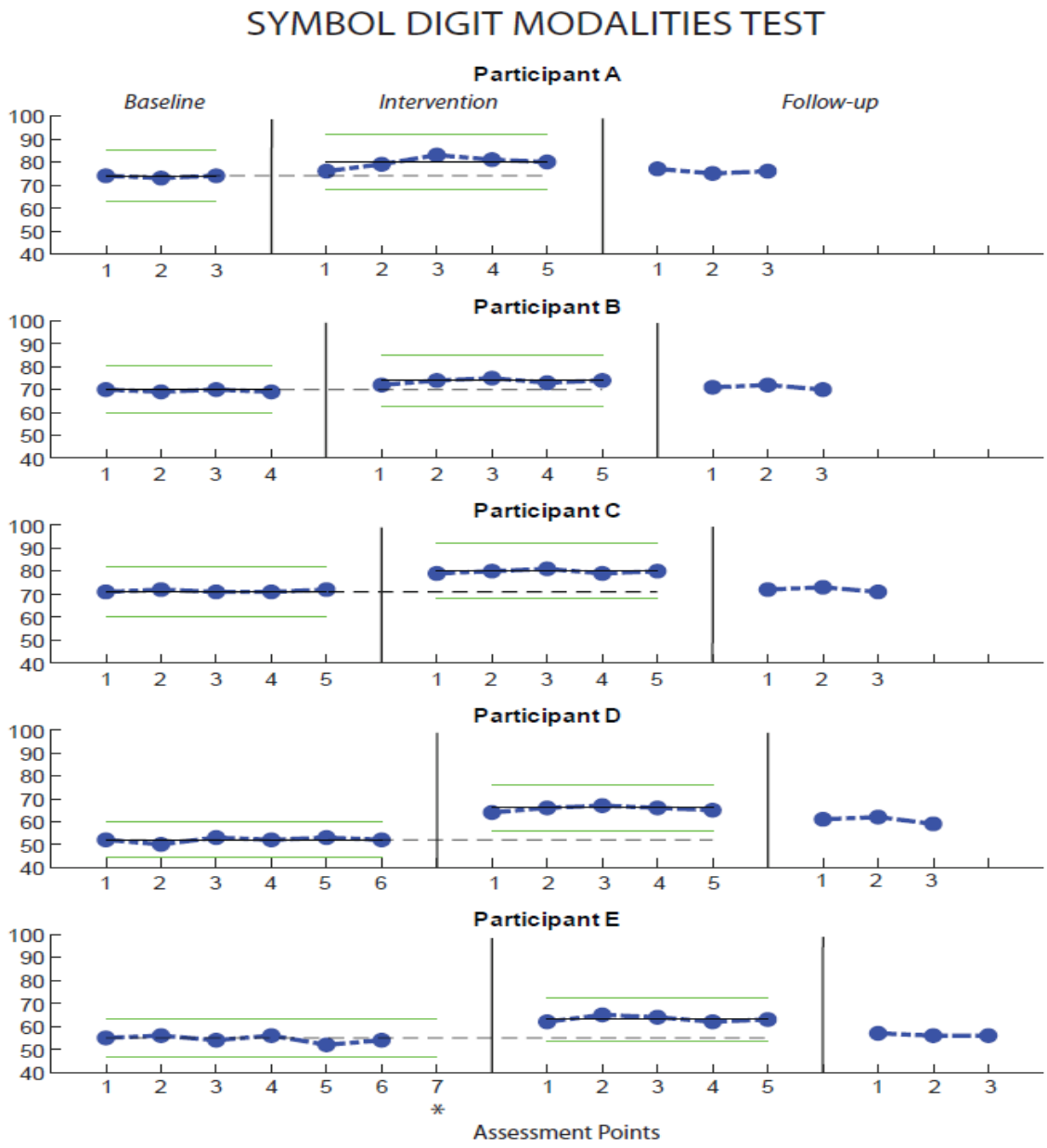


Figure 2.28: The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the scores of the tests are presented. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases only. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Figure 2.29. Visual representation of the Action Research Arm Test during baseline, intervention and follow-up phases – multiple baseline study.

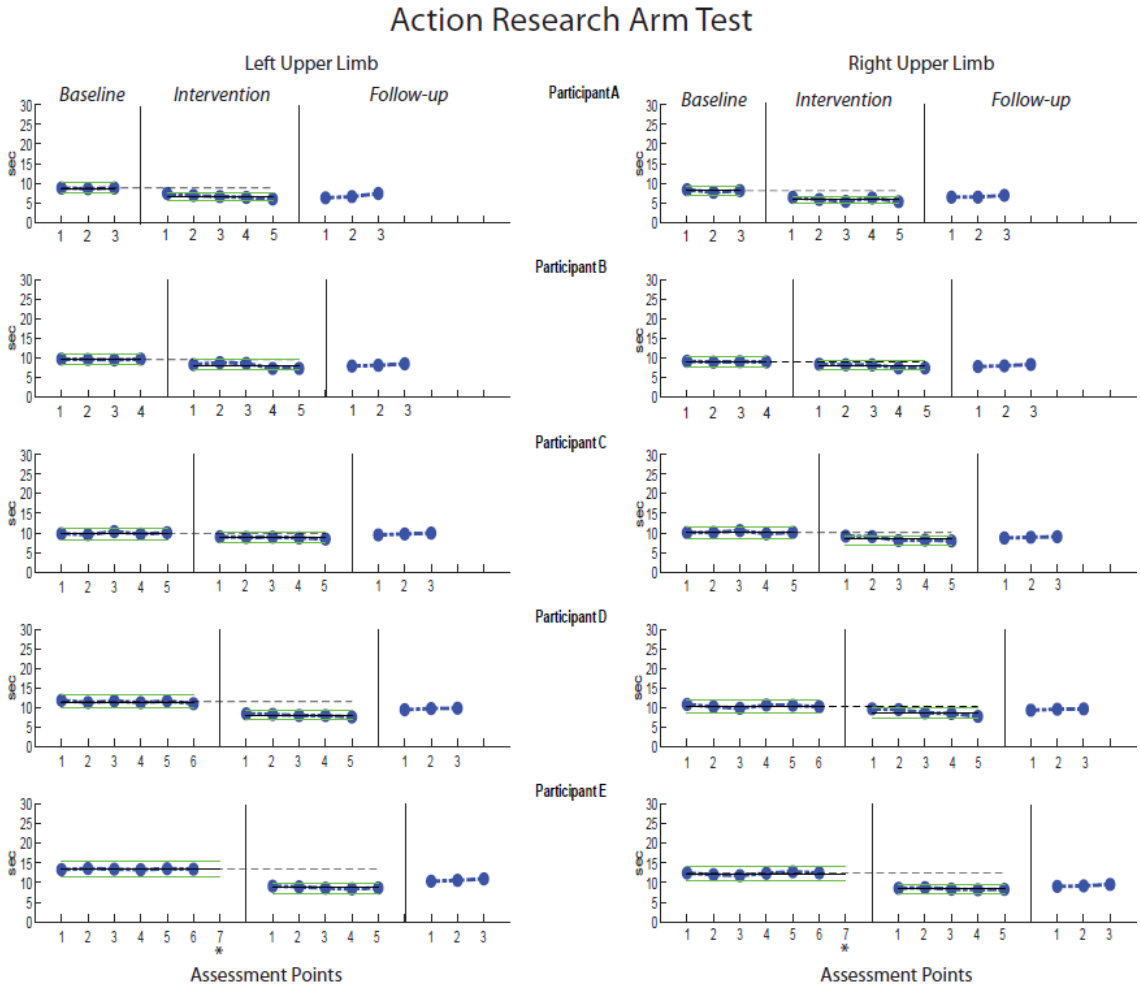


Figure 2.29: The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the scores (sec) of the tests are presented. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases only. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Figure 2.30. Visual representation of the Hand Grip Test during baseline, intervention and follow-up phases – multiple baseline study.

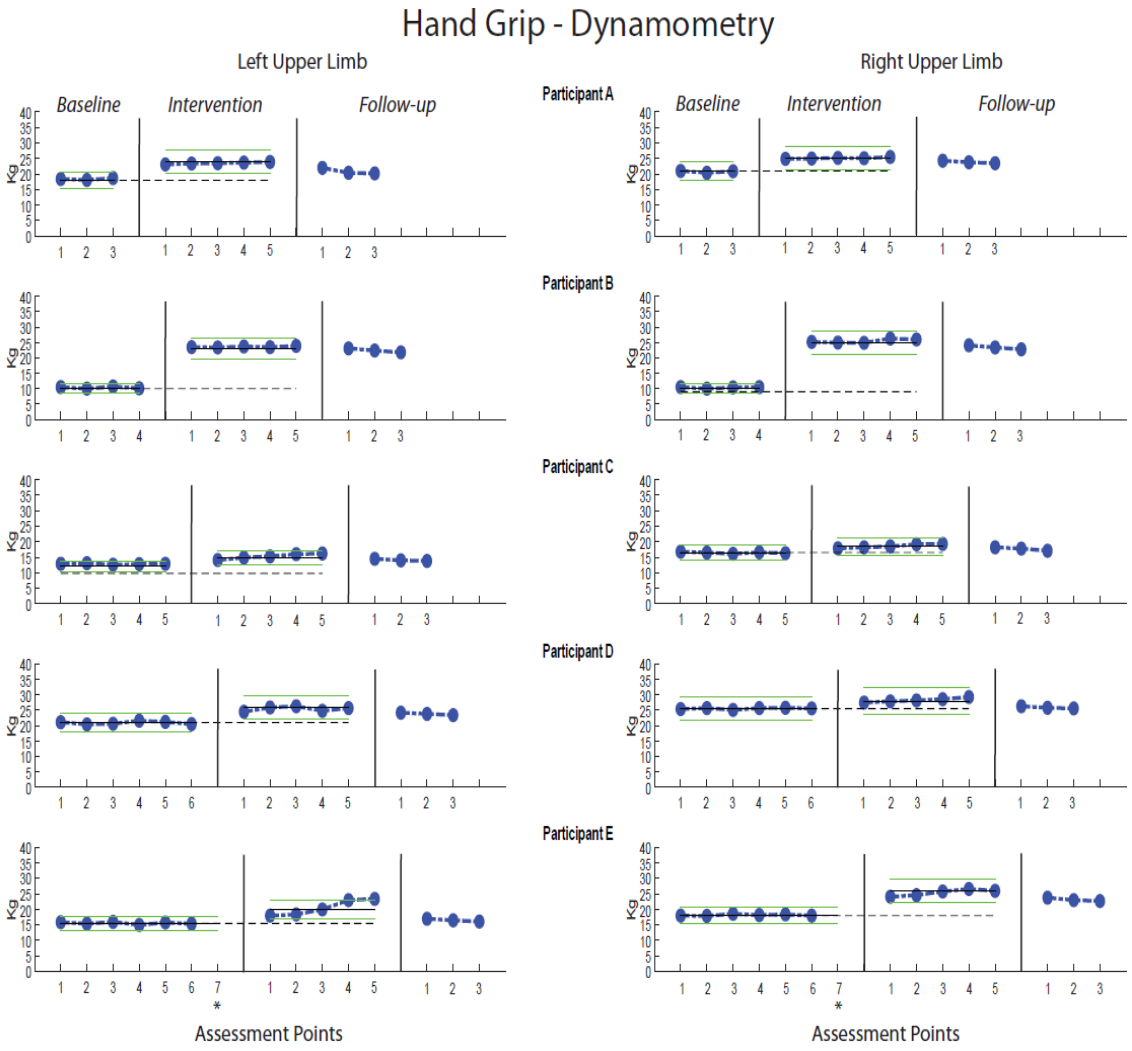


Figure 2.30: The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the values (kg) of the test are presented. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases only. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Figure 2.31. Visual representation of the Modified Fatigue Impact Scale during baseline, intervention and follow-up phases – multiple baseline study.

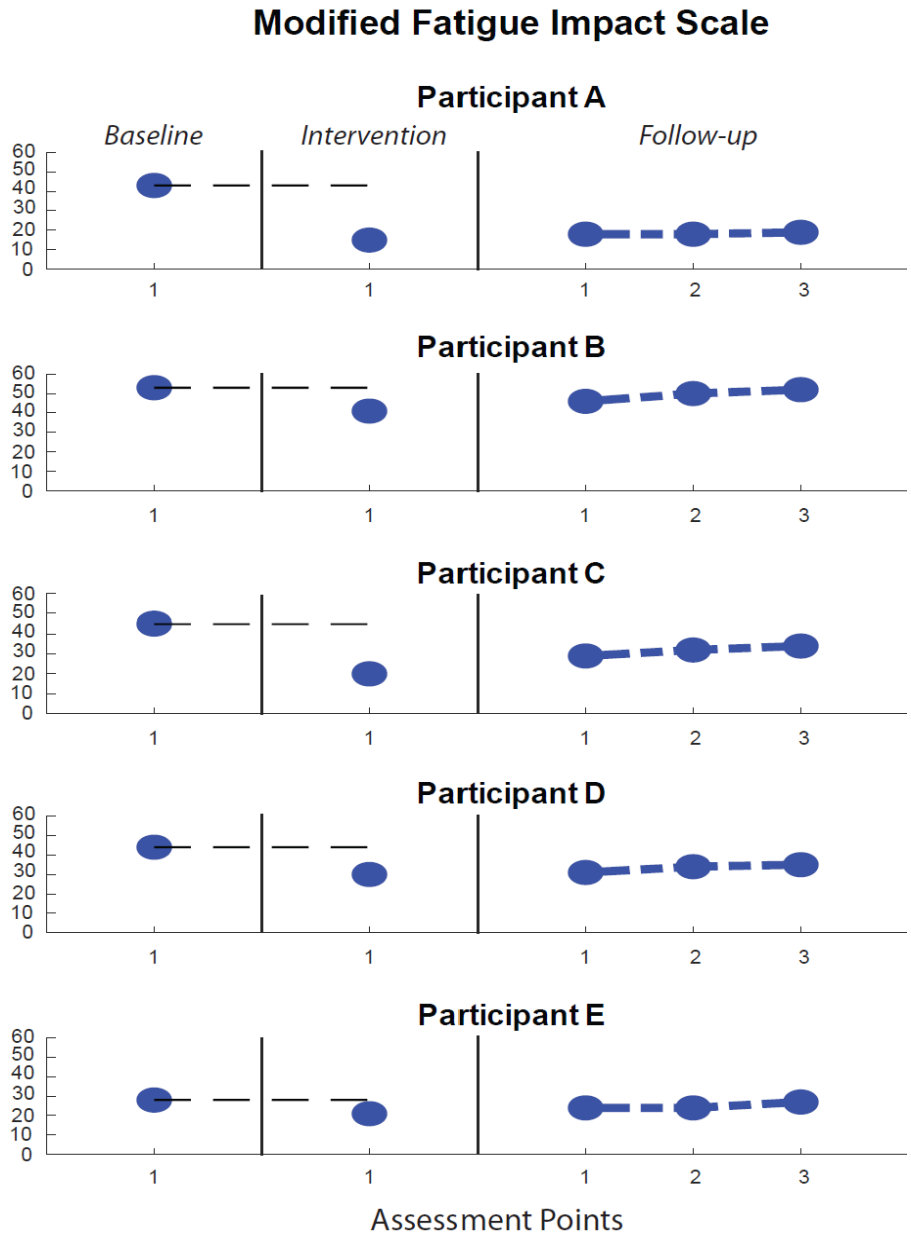


Figure 2.31: The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the scores of the tests are presented. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases only. The black horizontal lines represent the within-phase (i.e., intervention) mean.

Figure 2.32. Visual representation of the Medical Outcomes Study Questionnaire Short Form 36 Health Survey during baseline, intervention and follow-up phases – multiple baseline study.

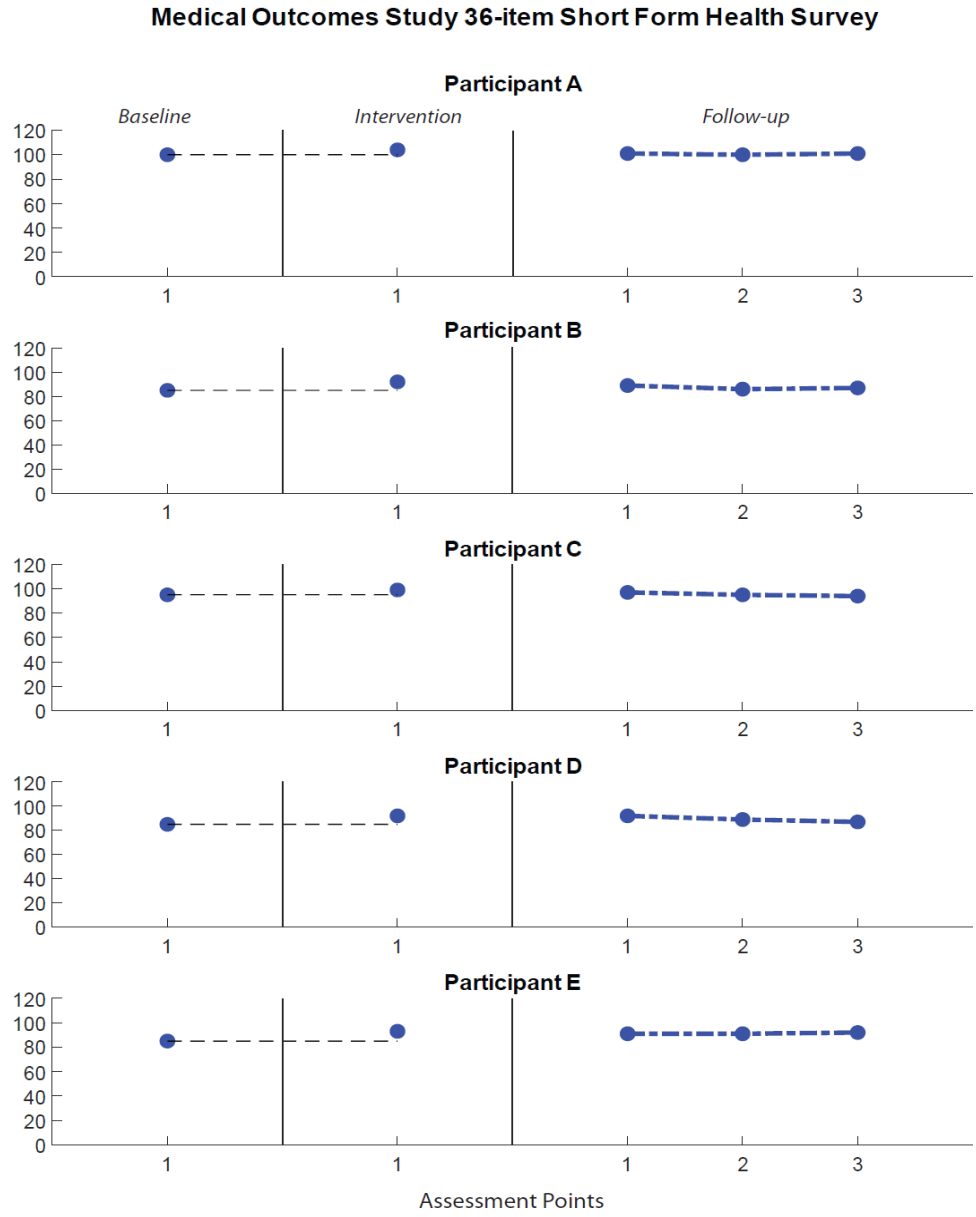


Figure 2.33: The number of assessment points per phase are presented on the x-axis, whereas on the y-axis the scores of the tests are presented. The vertical line between the data points indicate the two study phases (i.e., baseline, intervention). The area between the green lines refer to the acceptable range regarding the stability criterion (i.e., ±15% of the median of each phase (Lobo et al., 2017). The black horizontal dashed lines represent the PEM (MA, 2006), which has been calculated between the baseline and intervention phases only. The black horizontal lines represent the within-phase (i.e., intervention) mean.

2.4 Chapter Summary

To investigate the effects of a 12-week in-phase bilateral upper limb exercise protocol in pwRRMS, a clinical trial was conducted using a concurrent multiple baseline design across five participants. This study employed a more robust methodology after acknowledging the limitations of a previous single-case pilot study.

Contrary to study's expectations, the exercise protocol did not result in significant changes in CMCT, MEPs amplitude and latency. Following visual analysis of the CMCT, MEPs amplitude and latency measurements, no significant improvement (i.e., reduction) was observed to any of the participants, possibly due to high variability, low data stability and due to unexpected trend directions (i.e., upward/downward accordingly) across all study phases.

However, the intervention did have a notable impact on the rMT—a key indicator of cortical excitability and plasticity (Caramia et al., 2004; Hallett, 2007). This outcome implies that while the exercises did not alter neural conduction speed, they may have facilitated increased neural excitability. In addition to these neurophysiological insights, the protocol led to observable improvements in clinical measures. These enhancements spanned various domains, including motor function and cognitive processing, suggesting a broad therapeutic potential of the exercise regimen for pwRRMS.

The promising effects of the exercise regimen on neurophysiological and clinical measures, particularly in motor function and cognitive processing, underscore the potential in-phase bilateral exercises in managing RRMS. These findings hold significant relevance, considering that most pwRRMS will eventually transition to the progressive form of MS.

CHAPTER 3

Study on the Effects of In-Phase Bilateral Exercise in Progressive Multiple Sclerosis

3.1 Introduction

Given that most people with Relapsing-Remitting Multiple Sclerosis (pwRRMS) eventually transition to the Progressive MS (PMS), understanding the progression and treatment options for PMS becomes critical. This transition is associated with an increase in disability and a steady accumulation of neurological impairment. Due to the high prevalence of this progression, a substantial number of individuals are already affected by the progressive form of MS. This necessitates the development of targeted treatments for progressive MS, as existing therapies are less effective at this stage of the disease. In response to this need, a second clinical trial was conducted to focus on the second research question—investigating the effectiveness and safety of a new treatment or therapeutic strategy aimed specifically at halting or slowing the progression of this subtype. This clinical trial study builds on the previous clinical trial study of this thesis on pwRRMS and seeks to provide essential data for improving the quality of life (QoL) and long-term outcomes for people with PMS (pwPMS). The current clinical trial study was registered on ClinicalTrials.gov (NCT06436131) (Appendix X - Spirit Checklist_In-phase bilateral upper limb exercises in pwPMS).

PMS includes both Secondary Progressive MS and Primary Progressive MS. MS is a highly variable disease, with nearly 50% of patients initially diagnosed with RRMS. Over time, typically after 10 – 15 years, RRMS often transitions into Secondary Progressive MS, characterized by a gradual worsening of clinical symptoms. In approximately 15% of cases, MS follows a continuously progressive course from onset, known as Primary Progressive MS (Lublin & Reingold, 1996).

Since most pwRRMS eventually transition to PMS, understanding its progression and treatment options is crucial. This transition is associated with increased disability and a steady accumulation of neurological impairment. Given the high prevalence of this progression, a significant number of individuals are already living with PMS. However, current therapies are less effective at this stage, highlighting the need for the development of targeted treatments.

Cognitive Impairments and the Role of Bilateral Movement

PwPMS not only experience physical impairment but also commonly suffer from cognitive dysfunction (Højsgaard Chow et al., 2018), which significantly affects their

QoL. Information processing speed is one of the most commonly impaired cognitive functions (Benedict et al., 2020; DeLuca et al., 2020) in both Secondary Progressive MS and Primary Progressive MS (Ukkonen et al., 2009). Various cognitive rehabilitation programs (DeLuca et al., 2020; Tacchino et al., 2023) have shown efficacy in improving MS-related cognitive dysfunctions, such as learning, memory, attention and cognitive processing.

Additionally, evidence highlights the positive impact of physical exercise on cognitive function (Li et al., 2023; Orban et al., 2019; Sandroff, Motl, et al., 2016; Zimmer et al., 2018) and QoL (Beratto et al., 2024), in people with MS (pwMS). While cognitive and physical training are often combined, physical exercise provides unique advantages by being more engaging, accessible and holistic in managing both cognitive and physical symptoms, including fatigue, balance issues and muscle weakness.

Research also suggests a strong link between cognitive function and upper limb performance in both healthy populations and pwMS (Einarsson et al., 2006; Kierkegaard et al., 2012; Raats et al., 2018; Yozbatiran et al., 2006). This connection is supported by the dense projections from the anterior cingulate cortex to the motor cortex and spinal cord (Asemi et al., 2015; Bush et al., 2000; Paus, 2001), emphasizing the interdependent nature of motor and cognitive functions.

Bilateral Movement and Information Processing Speed

PwPMS often experience a decline in information processing speed (Benedict et al., 2020; Bergendal et al., 2007; DeLuca et al., 2020), which ultimately impacts manual dexterity (Poirier, 1988). This raises a fundamental question: Could in-phase bilateral upper limb exercises enhance information processing speed and, in turn, improve manual dexterity in this population?

Information processing speed refers to the ability to receive, process, and respond to information within a given time frame (Kail & Salthouse, 1994), while manual dexterity involves the coordinated control of fine and gross voluntary upper limb movements (Poirier, 2015). Impaired manual dexterity in pwMS significantly reduces their ability to perform activities of daily living (ADL) and participate in social interactions, leading to decreased independence and a lower QoL (Yozbatiran et al., 2006).

Several studies suggested that bilateral movement, which requires coordinated use of both body sides, plays a crucial role in enhancing cognitive functions, including information processing speed (Grefkes et al., 2008; Rudisch et al., 2020; Swinnen & Wenderoth, 2004; Vasylenko et al., 2018b). Bilateral movements have been found to strengthen interhemispheric communication via the corpus callosum, facilitating efficient neural integration. Bimanual hand coordination is linked to symmetrical facilitation of neural activity through increased intrahemispheric connectivity and enhanced transcallosal coupling between the supplementary motor area (SMA) and the primary motor cortex (M1) (Asemi et al., 2015; Grefkes et al., 2008; Rudisch et al., 2020; Swinnen & Wenderoth, 2004). Notably, Grefkes et al. (2008), demonstrated that in-phase bilateral upper-limb movements significantly enhance interhemispheric connectivity between the SMA and M1 in both hemispheres. This improved connectivity facilitates better integration of sensory and motor information, ultimately enhancing information processing speed, a crucial factor in complex cognitive tasks that require quick responses and adaptability (Grefkes et al., 2008).

Impairments in information processing speed are directly associated with other cognitive domains, such as working memory and attention (Genova et al., 2012; Leavitt et al., 2011; Lengenfelder et al., 2006), as well as with various motor skills, including eye-hand coordination and manual dexterity. Evidence from previous studies in both healthy individuals and pwMS indicated a strong relationship between cognitive functions and upper limb performance (Einarsson et al., 2006; Kierkegaard et al., 2012; Raats et al., 2018; Yozbatiran et al., 2006). This relationship is supported by dense neural projections from the anterior cingulate cortex (Figure 1.19) to the motor cortex and spinal cord (Asemi et al., 2015; Bush et al., 2000; Paus, 2001).

Bilateral Movement and Cognitive Enhancement

Bilateral movement activates multiple neural pathways related to sensorimotor integration and cognitive function (Asemi et al., 2015; Grefkes et al., 2008; Rudisch et al., 2020; Swinnen & Wenderoth, 2004). When individuals perform movements requiring coordination between both sides of the body, motor control and executive function networks are stimulated, leading to more efficient cognitive processing and enhanced adaptability to new information (Alharthi & Almurdi, 2023; Fernandes et al., 2016).

Cognitive functions such as working memory and attention are closely tied to information processing speed (Genova et al., 2012; Leavitt et al., 2011; Lengenfelder et al., 2006). Bilateral movements have been shown to enhance executive function by increasing engagement of prefrontal cortex regions responsible for attention control and memory retention. Research suggests that movement-based interventions, including rhythmic coordination drills and structured exercise programs, improve cognitive performance in children (Buchele Harris et al., 2018). By reinforcing neural circuits related to attention and memory, bilateral movement supports faster information retrieval and overall cognitive efficiency (Koper et al., 2024). These findings highlight the importance of incorporating bilateral movement into therapeutic interventions to optimize cognitive function across the lifespan.

Based on these evidences and building upon the first clinical trial of this thesis (Sokratous et al., 2023) (see Chapter 2.3), which demonstrated that in-phase bilateral upper limb exercises significantly improved information processing speed in five individuals with RRMS, the current study investigates whether similar benefits extend to pwPMS. Previous studies (Koeneke et al., 2004; Swinnen, 2002; Temprado et al., 1999) have shown that in-phase bilateral movements require less attentional load and motor control compared to unilateral or other types of bilateral coordination. This efficiency makes them particularly suitable for targeted exercise interventions.

The primary aim of this study is to test the hypothesis that a 12-week group exercise program focused on in-phase bilateral upper limb movements can improve information processing speed in pwPMS compared to conventional exercises such as balance training, core strengthening, and treadmill walking (Edwards & Pilutti, 2017; Moon et al., 2013). A secondary aim is to determine whether improvements in information processing speed also lead to enhanced manual dexterity in this clinical population.

The Role of Group Exercise in Cognitive and Physical Health

Group exercise programs have been shown to be more effective than individual exercise sessions in improving cognitive function, particularly in older adults and those at risk for cognitive decline. Research suggests that group exercise fosters social interaction, which not only enhances motivation but also stimulates brain regions associated with social cognition and executive functions (Karamacoska et al., 2023; Yuan et al., 2024). The

communal aspect of group exercise creates an engaging environment, improving adherence and intensity—both crucial for cognitive benefits (Mandolesi et al., 2018).

Additionally, group exercise programs offer a structured approach that integrates aerobic and resistance training, contributing to improved neuroplasticity and brain function (Yuan et al., 2024). These interventions have been linked to reduced cognitive decline and improved neurovascular health, making them particularly beneficial for individuals at risk of Alzheimer's disease and other dementias (Karamacoska et al., 2023).

Therefore, this study aims to provide critical insights into the role of in-phase bilateral upper limb exercises in improving information processing speed and manual dexterity in pwPMS. By leveraging the benefits of structured group exercise, this research seeks to enhance cognitive function, physical health, and overall QoL in this clinical population.

3.1.1 Participants

All participants were patients registered in the outpatient registry at The Cyprus Institute of Neurology and Genetics. Each participant has a unique patient file containing detailed examinations and clinical descriptions, as they receive regular medical appointments and evaluations by neurologists and other healthcare providers at the institute. Therefore, all inclusion and exclusion criteria were confirmed by a neurologist who reviewed the individual medical records. The inclusion criteria were as follows: 1) diagnosis of PMS (Primary Progressive MS or/and Secondary Progressive MS), 2) Expanded Disability Status Scale (EDSS) score between three and six (Kurtzke, 1983), no relapse within the last 30 days, 4) aged between 30 and 70 years and 5) Mini-Mental State Examination score between 20 and 30 (mild to no cognitive impairment) (Folstein et al., 1975). The exclusion criteria included 1) history of any disease affecting the central nervous system other than MS (e.g., stroke, Parkinson's disease, cerebral palsy), 2) history of cardiovascular disease (e.g., known aneurism, myocardial infarction, hyper/hypotension, heart failure), 3) severe orthopedic disorders (e.g., knee or hip replacement, spondylosurgery, disk herniation, recent bone fracture), 4) mental disorders (e.g., depression, schizophrenia, bipolar syndrome), 5) pregnancy during the implementation of the study timeline, 6) hearing impairments (i.e., deafness), 7) visual deficit (e.g., optic neuritis, blindness, diplopia, glaucoma, blurred vision), 8) history of epileptic seizures

and 9) spasticity level on upper or lower limbs more than 1+ (slight increase in muscle tone) on the Modified Ashworth Scale (Meseguer-Henarejos et al., 2018).

3.1.2 Participants recruitment

Regarding recruitment of participants, an information letter was given to the outpatients of The Cyprus Institute of Neurology and Genetics between May 2023 and August 2023, to express their interest participating in the current study. A total of 28 patients (n=28) expressed interest to participate. The enrollment was performed by the neurologist at The Cyprus Institute of Neurology and Genetics during September 2023, in which eight patients (n=8) were excluded according to the exclusion criteria (Figure 3.1). Between September 25 and 30, 2023, the participants' medical records were collected and the assignment for all 20 participants (n=20) was completed. To ensure that authors couldn't identify individual participants during or after data collection, they did not have access to participants' documents, apart from the principal investigator (DS). The study took place from October 2 to December 22, 2023.

The 1:1 allocation of participants into the two groups was conducted by an independent researcher who was blinded to the participants' clinical condition. The researcher organized the allocation based on individual EDSS score (Kurtzke, 1983), age, gender and hand dominancy to ensure the homogeneity of the groups. These four parameters were chosen as the criteria for the group allocation, due to their impact in exercise performance and due to the direct relationship with the study's outcome measures. As far as we know, increased disability level (i.e., EDSS) (Marck et al., 2014) and the age of participants (Berthelot et al., 2019) negatively impact exercise performance. Also, gender is an important parameter affecting exercise performance, with women generally exhibiting lower performance levels than men (Hunter & Senefeld, 2024). Lastly, because our research hypothesis is based on bilateral hand dexterity, the criterion of hand dominancy could have an impact in the study's results.

Figure 3.1. Group comparison study - Participants' flowchart.

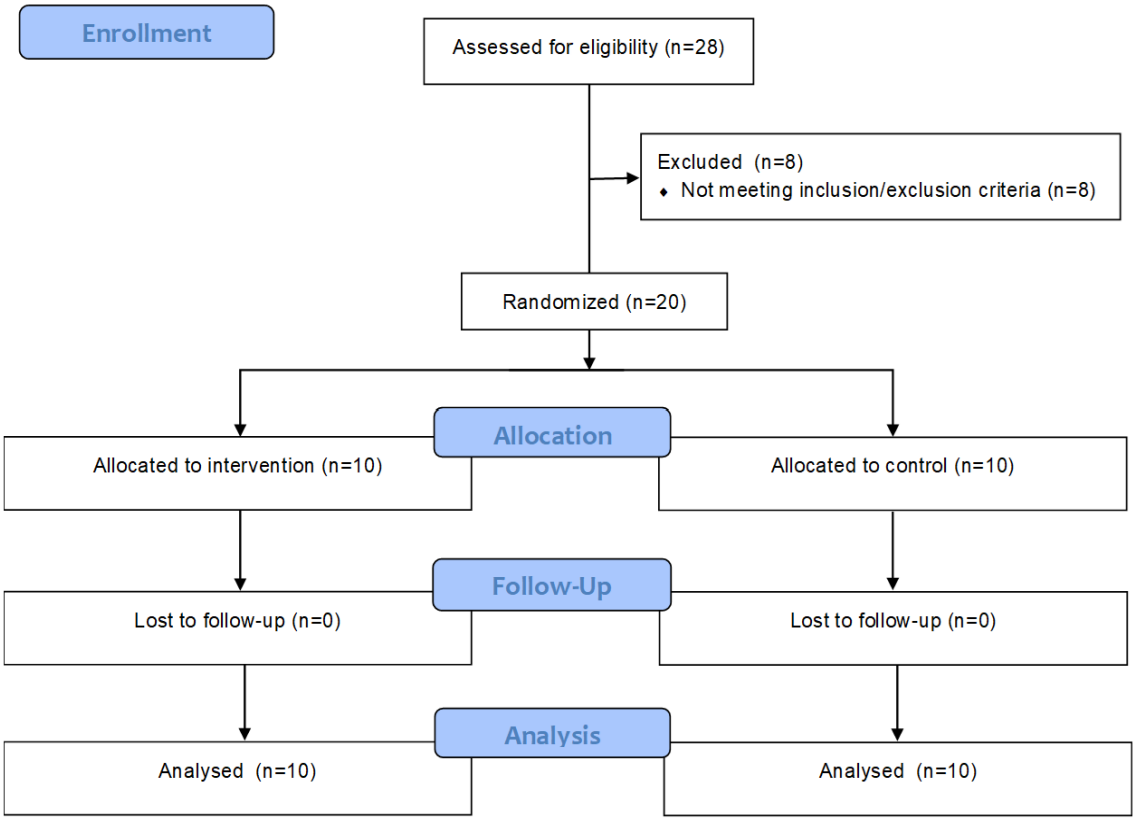


Figure 3.1: Eight participants did not meet the inclusion criteria, therefore were not enrolled in the study. Enrolled participants were allocated to experimental (n=10) and active control (n=10) group. All of the participants completed the study and none of them was excluded from the data analysis.

The allocation process for the participants' assignment was performed via Microsoft Excel 2021 software following the steps below. Firstly, the principal investigator (DS) created a list of participants by a unique identifier (Figure 3.2a). Secondly, the independent researcher categorized all participants according to the individual EDSS score (Figure 3.2b). Thirdly, the participants were manually placed alternately in the two groups, so that there were equal numbers of participants in each group based on the EDSS score (Figure 3.2c). Fourthly, the participants in both groups were re-allocated based on the individual age, so that the mean age is similar between the two groups and at the same time have similar mean EDSS score between the two groups (Figure 3.2d). The next step was again a re-allocation of the participants in both groups based on gender, so that the female to male ratio is similar between the two study groups and retain equal mean EDSS score and mean age between the groups (Figure 3.2e). Finally, the participants in both

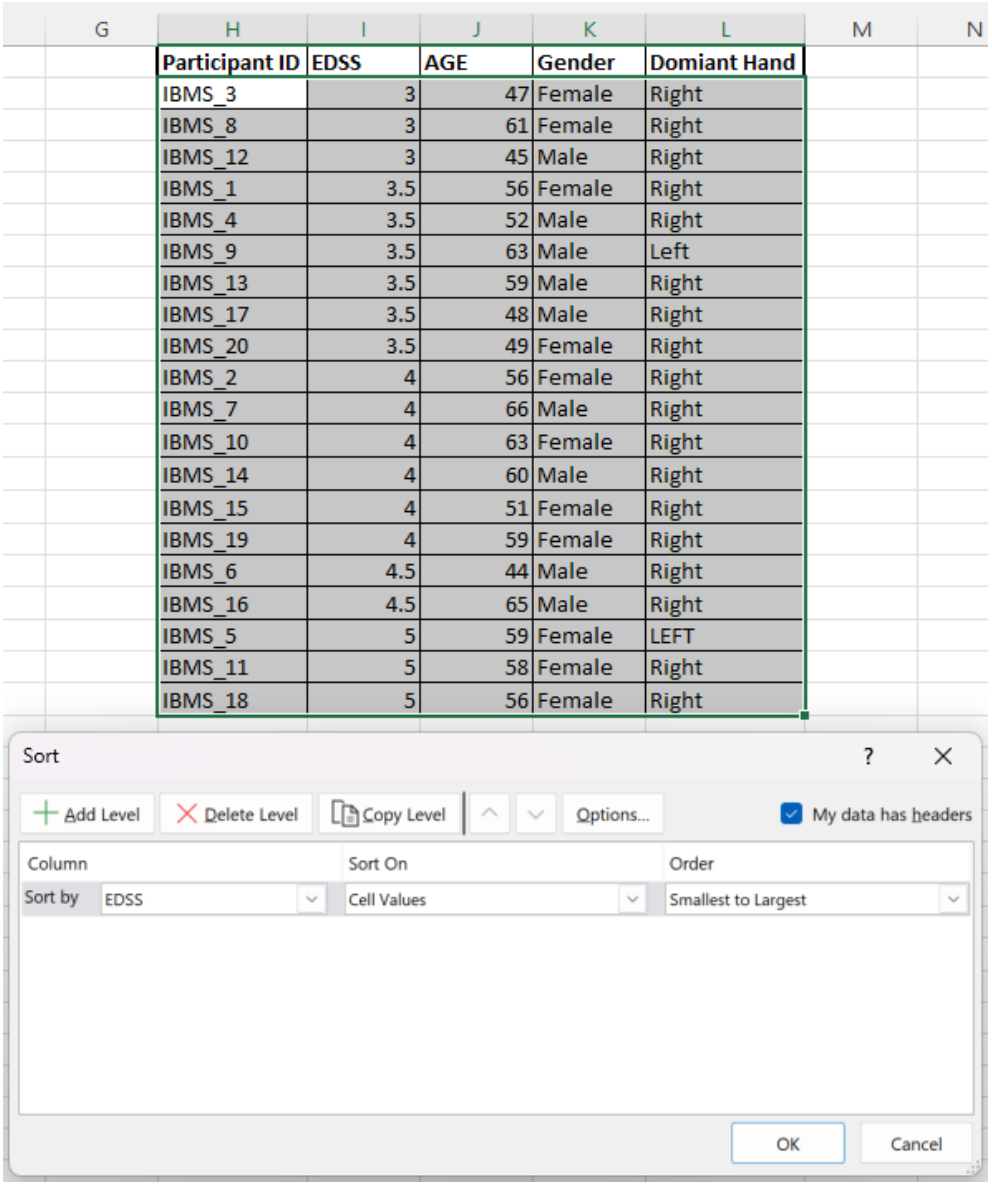
groups were re-allocated based on hand dominancy, so that the left to right handed ratio is similar between the two study groups while retaining similar mean EDSS score, mean age and female to male ratio between the groups (Figure 3.2f). Following these steps, we ensured the homogeneity of the two study groups and we achieved similar group mean of the EDSS score (Experimental group = 3.85; Moderate to significant disability, Active control group = 3.95; Moderate to significant disability), age (Experimental group = 55.8 years old, Active control group = 3.95 years old), female to male ratio (Experimental group: Female = 6 Male = 4, Active control group: Female = 5 Male = 5) and left to right handed ratio (Experimental group: Right = 9 Left = 1, Active control group: Right = 9 Left = 1).

Figure 3.2a. Participants list prior randomization process.

	A	B	C	D	E
1	**Participant ID**	**EDSS**	**AGE**	**Gender**	**Domiant Hand**
2	IBMS_1	3.5	56	Female	Right
3	IBMS_2	4	56	Female	Right
4	IBMS_3	3	47	Female	Right
5	IBMS_4	3.5	52	Male	Right
6	IBMS_5	5	59	Female	Left
7	IBMS_6	4.5	44	Male	Right
8	IBMS_7	4	66	Male	Right
9	IBMS_8	3	61	Female	Right
10	IBMS_9	3.5	63	Male	Left
11	IBMS_10	4	63	Female	Right
12	IBMS_11	5	58	Female	Right
13	IBMS_12	3	45	Male	Right
14	IBMS_13	3.5	59	Male	Right
15	IBMS_14	4	60	Male	Right
16	IBMS_15	4	51	Female	Right
17	IBMS_16	4.5	65	Male	Right
18	IBMS_17	3.5	48	Male	Right
19	IBMS_18	5	56	Female	Right
20	IBMS_19	4	59	Female	Right
21	IBMS_20	3.5	49	Female	Right

Figure 3.2a: ID; Identity, EDSS; Expended Disability Status Scale. Participants' list, in which each participant was listed by a unique identifier. Individual EDSS score, age, gender and hand dominancy are presented in deferent columns.

Figure 3.2b. Participants' list according EDSS score.



Participant ID	EDSS	AGE	Gender	Domiant Hand
IBMS_3	3	47	Female	Right
IBMS_8	3	61	Female	Right
IBMS_12	3	45	Male	Right
IBMS_1	3.5	56	Female	Right
IBMS_4	3.5	52	Male	Right
IBMS_9	3.5	63	Male	Left
IBMS_13	3.5	59	Male	Right
IBMS_17	3.5	48	Male	Right
IBMS_20	3.5	49	Female	Right
IBMS_2	4	56	Female	Right
IBMS_7	4	66	Male	Right
IBMS_10	4	63	Female	Right
IBMS_14	4	60	Male	Right
IBMS_15	4	51	Female	Right
IBMS_19	4	59	Female	Right
IBMS_6	4.5	44	Male	Right
IBMS_16	4.5	65	Male	Right
IBMS_5	5	59	Female	LEFT
IBMS_11	5	58	Female	Right
IBMS_18	5	56	Female	Right

Figure 3.2b: ID; Identity, EDSS; Expended Disability Status Scale. To categorize the participants, list according to the EDSS score, we sorted the entire table by the column I which refers to the EDSS, in Microsoft Excel 2021. Firstly, all columns must be highlighted, then from the "Data" tab we selected the option "Sort" and we chose the option sort by "EDSS", from "smallest to largest" order. Therefore, the participation list was sorted in an ascending order according to the individual EDSS score.

Figure 3.2c. Allocation of participants according to EDSS score.

N	O	P	Q	R
Experimental Group				
Participant ID	**EDSS**	**AGE**	**Gender**	**Domiant Hand**
IBMS_3	3	47	Female	Right
IBMS_12	3	45	Male	Right
IBMS_4	3.5	52	Male	Right
IBMS_13	3.5	59	Male	Right
IBMS_20	3.5	49	Female	Right
IBMS_7	4	66	Male	Right
IBMS_14	4	60	Male	Right
IBMS_19	4	59	Female	Right
IBMS_16	4.5	65	Male	Right
IBMS_11	5	58	Female	Right
Mean	**3.8**			
Active Control Group				
Participant ID	**EDSS**	**AGE**	**Gender**	**Domiant Hand**
IBMS_8	3	61	Female	Right
IBMS_1	3.5	56	Female	Right
IBMS_9	3.5	63	Male	Left
IBMS_17	3.5	48	Male	Right
IBMS_2	4	56	Female	Right
IBMS_10	4	63	Female	Right
IBMS_15	4	51	Female	Right
IBMS_6	4.5	44	Male	Right
IBMS_5	5	59	Female	Left
IBMS_18	5	56	Female	Right
Mean	**4**			

Figure 3.2c: ID; Identity, EDSS; Expended Disability Status Scale. After the participants list was sorted in an ascending order according to the individual EDSS score, all participants were manually placed alternately in the two groups, so that there were equal numbers of participants in each group based on the EDSS score and also to have equal group mean EDSS score between the groups.

Figure 3.2d. Allocation of participants according to age.

T	U	V	W	X
Experimental Group				
Participant ID	**EDSS**	**AGE**	**Gender**	**Domiant Hand**
IBMS_3	3	47	Female	Right
IBMS_12	3	45	Male	Right
IBMS_4	3.5	52	Male	Right
IBMS_13	3.5	59	Male	Right
IBMS_20	3.5	49	Female	Right
IBMS_7	4	66	Male	Right
IBMS_14	4	60	Male	Right
IBMS_19	4	59	Female	Right
IBMS_16	4.5	65	Male	Right
IBMS_11	5	58	Female	Right
Mean	**3.8**	**56**		
Active Control Group				
Participant ID	**EDSS**	**AGE**	**Gender**	**Domiant Hand**
IBMS_8	3	61	Female	Right
IBMS_1	3.5	56	Female	Right
IBMS_9	3.5	63	Male	Left
IBMS_17	3.5	48	Male	Right
IBMS_2	4	56	Female	Right
IBMS_10	4	63	Female	Right
IBMS_15	4	51	Female	Right
IBMS_6	4.5	44	Male	Right
IBMS_5	5	59	Female	Left
IBMS_18	5	56	Female	Right
Mean	**4**	**55.7**		

Figure 3.2d: ID; Identity, EDSS; Expended Disability Status Scale. After the participants were allocated in the two study groups according to the EDSS score, all participants were re-allocated manually in the two groups, so that there were equal numbers of participants with equal group mean age. Parallel to the age, still we maintain to have equal group mean EDSS score between the groups.

Figure 3.2e. Allocation of participants according to gender.

Z	AA	AB	AC	AD
Experimental Group				
Participant ID	**EDSS**	**AGE**	**Gender**	**Domiant Hand**
IBMS_3	3	47	FEMALE	Right
IBMS_12	3	45	MALE	Right
IBMS_1	3.5	56	FEMALE	Right
IBMS_13	3.5	59	MALE	Right
IBMS_20	3.5	49	FEMALE	Right
IBMS_7	4	66	MALE	Right
IBMS_14	4	60	MALE	Right
IBMS_19	4	59	FEMALE	Right
IBMS_5	5	59	FEMALE	Left
IBMS_11	5	58	FEMALE	Right
Mean	**3.85**	**55.8**	**Total Females = 6**	
			Total Males = 4	
Active Control Group				
Participant ID	**EDSS**	**AGE**	**Gender**	**Domiant Hand**
IBMS_8	3	61	FEMALE	Right
IBMS_4	3.5	52	MALE	Right
IBMS_9	3.5	63	MALE	Left
IBMS_17	3.5	48	MALE	Right
IBMS_2	4	56	FEMALE	Right
IBMS_10	4	63	FEMALE	Right
IBMS_15	4	51	FEMALE	Right
IBMS_6	4.5	44	MALE	Right
IBMS_16	4.5	65	MALE	Right
IBMS_18	5	56	FEMALE	Right
Mean	**3.95**	**55.9**	**Total Females = 5**	
			Total Males = 5	

Figure 3.2e: ID; Identity, EDSS; Expended Disability Status Scale. After the participants were allocated in the two study groups according to the EDSS score and age, all participants were re-allocated in the two groups based on gender. Compared to the previous allocation (Figure 3.2d), there was an unequal female to male ratio, therefore we manually placed the participants in the two groups so that there were equal numbers of participants with equal female to male ratio (highlighted). Parallel with the gender ratio, still we maintain to have equal group mean EDSS score and age between the groups.

Figure 3.2f. Allocation of participants according to hand dominancy.

AF	AG	AH	AI	AJ
Experimental Group				
Participant ID	**EDSS**	**AGE**	**Gender**	**Domiant Hand**
IBMS_3	3	47	FEMALE	Right
IBMS_12	3	45	MALE	Right
IBMS_1	3.5	56	FEMALE	Right
IBMS_13	3.5	59	MALE	Right
IBMS_20	3.5	49	FEMALE	Right
IBMS_7	4	66	MALE	Right
IBMS_14	4	60	MALE	Right
IBMS_19	4	59	FEMALE	Right
IBMS_5	5	59	FEMALE	Left
IBMS_11	5	58	FEMALE	Right
Mean	**3.85**	**55.8**	**Total Females = 6**	**Total Right = 9**
			Total Males = 4	**Total Left = 1**
Active Control Group				
Participant ID	**EDSS**	**AGE**	**Gender**	**Domiant Hand**
IBMS_8	3	61	FEMALE	Right
IBMS_4	3.5	52	MALE	Right
IBMS_9	3.5	63	MALE	Left
IBMS_17	3.5	48	MALE	Right
IBMS_2	4	56	FEMALE	Right
IBMS_10	4	63	FEMALE	Right
IBMS_15	4	51	FEMALE	Right
IBMS_6	4.5	44	MALE	Right
IBMS_16	4.5	65	MALE	Right
IBMS_18	5	56	FEMALE	Right
Mean	**3.95**	**55.9**	**Total Females = 5**	**Total Right = 9**
			Total Males = 5	**Total Left = 1**

Figure 3.2f: ID; Identity, EDSS; Expended Disability Status Scale. After the participants were allocated in the two study groups according to the EDSS score, age and gender, all participants were re-allocated in the two groups base on hand dominancy. However, because from the previous allocation (Figure 3.2e), the same ratio between left and right handed was observed with equal group mean EDSS score and age between the groups, no re-allocation was performed.

All participants were instructed to maintain their usual daily routines and to refrain from participating in any other exercise programs during the study. They were also advised to continue taking their prescribed medications as usual throughout the study duration. Before recruitment, all participants reviewed and signed a written informed consent form. The study procedures were approved by the Cyprus National Bioethics Committee (ΕΕΒΚ/ΕΠ/2022/32) and conducted in accordance with ethical guidelines.

3.1.3 Study design

This study was a two-arm, double-blind randomized controlled trial designed to evaluate the effects of a 12-week exercise program focusing on in-phase bilateral upper limb movements on information processing speed in pwPMS (Primary Progressive MS and/or Secondary Progressive MS). To meet the study objectives, participants were allocated to one of two independent groups: the experimental group or the active control group.

A fitness instructor administered the exercise protocol for the experimental group, while two physiotherapists were recruited: one to oversee the exercises for the active control group and the other to conduct all clinical assessments. The experimental group participated in an exercise program based on in-phase bilateral upper limb movements, whereas the active control group followed a program of conventional exercises (detailed in the intervention phase description below) (Figure 3.3).

To minimize interaction or influence between groups, participants exercised separately, with no contact between members of different groups during sessions. Participants were blinded to their group assignments and were unaware of whether they belonged to the experimental or active control group. Similarly, the fitness instructor and physiotherapist administering the exercise programs were blinded to group assignments and did not know which group was designated as experimental or control. However, the principal investigator (DS), responsible for data collection, was aware of the participants' group allocations. Demographic and baseline clinical characteristics of all participants were collected prior to the intervention and are presented in Table 3.1.

Table 3.1. Participants' demographic and baseline clinical characteristics.

Participant	**EDSS**	**Age (years)**	**Gender**	**Dominant hand**	**Disease duration**	**MMSE**
Experimental Group						
1	3	47	Female	Right	12	30
2	3	45	Male	Right	18	30
3	3.5	56	Female	Right	10	29
4	3.5	59	Male	Right	13	28

5	3.5	49	Female	Right	15	30
6	4	66	Male	Right	9	26
7	4	60	Male	Right	23	28
8	4	59	Female	Right	17	30
9	5	59	Female	Left	11	26
10	5	58	Female	Right	22	27
Active Control Group						
1	3	61	Female	Right	20	29
2	3.5	52	Male	Right	11	28
3	3.5	63	Male	Left	11	29
4	3.5	48	Male	Right	12	30
5	4	56	Female	Right	9	30
6	4	63	Female	Right	16	30
7	4	51	Female	Right	14	28
8	4.5	44	Male	Right	13	26
9	4.5	65	Male	Right	12	30
10	5	56	Female	Right	10	29

Table 3.1: EDSS; Expanded Disability Status Scale, MMSE; Mini Mental State Examination. The mean EDSS score for the experimental group was 3.85, indicating moderate to significant disability, while the active control group had a mean score of 3.95, also reflecting moderate to significant disability (Kurtzke, 1983). In terms of gender distribution, the experimental group had a female-to-male ratio of 6:4, whereas the active control group had an equal ratio of 5:5. Handedness was similar across groups, with nine out of ten participants in each group being right-handed. The mean age was 55.8 years for the experimental group and 55.9 years for the active control group. The mean Mini Mental State Examination score for the experimental group was 28.4, indicating no cognitive impairment, while the active control group had a mean Mini Mental State Examination score of 28.9, also reflecting no cognitive impairment (Folstein et al., 1975).

Figure 3.3. Timeline and schematic representation of the group comparison study design.

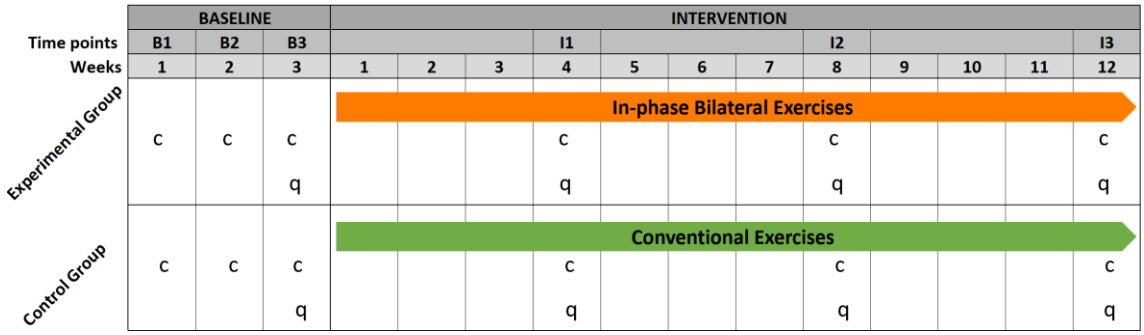


Figure 3.3: B; baseline, I; intervention, c; clinical assessments, q; questionaries. The first row refers to the study phases (i.e., baseline and intervention). The first row outlines the study phases (i.e., baseline and intervention). The second row indicates the time points for data collection, while the third row represents the study weeks. The study lasted a total of 15 weeks, with the first three weeks dedicated to the baseline phase and the remaining 12 weeks to the intervention phase. During the intervention phase, participants in the experimental group followed an exercise program focusing on in-phase bilateral upper limb movements (highlighted in orange), while those in the active control group participated in a program of conventional exercises (highlighted in green).

Baseline

All participants began the baseline phase simultaneously, which lasted for a period of three weeks. During the baseline phase, all participants were assessed at three different time points, once per week, on the outcome measures battery (see description of outcome measures below). During the three assessment points all participants were evaluated on cognitive and motor outcome measures (see description of primary and secondary outcomes below), except from the two subjective questionnaires (see secondary outcomes) which were completed once from each participant during the third week of the baseline phase (Figure 3.3).

Intervention

The intervention phases for both the experimental and active control groups lasted 12 consecutive weeks. Participants in the experimental group completed the exercise program (see page 146) three times per week, while participants in the active control group engaged in the same program once per week. For ethical reasons, we did not prohibit participants in the active control group from engaging in physical activity,

although a completely inactive control group would provide ideal conditions for comparison. Instead, we chose to provide the active control group with the minimum recommended level of exercise as outlined by the Cyprus General Health System (i.e., once a week). Many patients, for various reasons (e.g., most commonly due to economic constraints), are unable to afford private physiotherapy sessions beyond the minimum recommended frequency, as previously mentioned. Therefore, this was one of the main reasons the active control group was assigned to perform the exercises at this specific frequency (once a week), ensuring that the group had realistic parameters.

Experimental Group

All exercise sessions for the experimental group were conducted in a sports hall under the supervision of the same fitness instructor. Each session lasted approximately 60 minutes, and participants were required to complete at least 27 out of 36 sessions (75%) for their data to be included in the analysis (Akbar et al., 2020; Sokratous et al., 2023).

Each session consisted of three main components:

1. A five-minute warm-up involving full-body range-of-motion exercises.
2. The main exercise program, which focused on in-phase bilateral movements.
3. A five-minute cooldown, including passive stretching exercises targeting the muscle groups engaged during the session.

The experimental group followed a structured program emphasizing in-phase bilateral upper-limb movements, adapted to various sports activities and functional fitness exercises (see page 146). This program was organized as group circuit training (Figure 3.4), with all participants performing the exercises simultaneously during each session, adhering to MS exercise recommendations (Kalb et al., 2018; Kim et al., 2019).

Figure 3.4. Indoor exercise room.

Figure 3.4: Indoor exercise room of the experimental group. During all exercise sessions there was a continuous supervision of the fitness instructor.

According to the American College of Sports and Medicine guidelines and Kalb at al. (2020) (Table 3.2), pwMS are encouraged to engage in at least 150 minutes of exercise per week. This should include two to three sessions per week focusing on large muscle groups, with adequate rest between sets. When prescribing exercise protocols for pwMS, it is essential to consider MS-specific characteristics, safety, and gradual progression.

Table 3.2. Exercise recommendation for people with Multiple Sclerosis.

Recommended exercise	
EDSS 0 – 4.5 (mild impairments)	
Aerobic	- 2 – 3x/week, 10–30 minutes at a moderate exercise intensity (40% – 60% of HRmax or aerobic capacity), 3 – 4 RPE (on a 10-point RPE) - Cycle ergometer (upper limbs, lower limbs or combined) - Treadmill or overground walking - Sport activities - Aquatic therapy - Group-based exercises (target to individual limitations).
Advanced aerobic	- 5x/week - up to 40 minutes - 70% of peak aerobic capacity or 80% of maximum HR

	- Up to 7 RPE (on a 10-point RPE) - Cycle ergometer (upper limbs, lower limbs or combined) - Treadmill or overground walking - Sport activities, Aquatic therapy, Running, road cycling, walking - Group-based exercises (target to individual limitations).
Resistance	- 2 – 3x/week, 1–3 sets for each exercise, 8 – 15 repetitions/set, 5 – 10 exercises - Body weight exercises, free weights, resistance bands - Close-chain exercises are preferred.
Flexibility	- Daily, 2 – 3 sets of each stretch - Static stretching, yoga, clinical Pilates. - Group-based exercises (target to individual limitations).
Neuromotor	- 3 – 6x/week, 20 – 60 minutes - Interventions individualized for intensity and duration, targeting fall prevention, postural stability, coordination - Dance therapy, clinical yoga, Tai chi, hippotherapy, virtual reality, balance and motor control training, Sport activities, Aquatic therapy, group-based activities.
EDSS 5 – 6.5 (increasing mobility impairments)	
Aerobic **Resistance** **Flexibility** **Neuromotor**	Same as EDSS 0 – 4.5
Adaptive exercise	- 3 – 6 RPE (on a 10-point RPE) - Recumbent hand-cycle or three-wheel bike for cycling - Aerobic: heat sensitivity in some patients may require cooling interventions - Resistance: functional/multi-joint movements (sit-to-stand, stair climbing, reaching); neuromuscular electrical stimulation.

Table 3.2: EDSS; Expanded Disability Status Scale, HR; Heart Rate, RPE; Rate of Perceived Exertion. According to the individual EDSS score, specific guidelines related to different types

of exercises are recommended to be performed either from health professionals, either form people with Multiple Sclerosis.

The exercise program structure was based on the first clinical trial of this thesis (see page 101) (Sokratous et al., 2023). Each session included three sets of nine exercises targeting large upper-limb muscle groups (e.g., shoulder flexors, extensors, rotators, abductors, adductors; elbow flexors and extensors; horizontal abductors and adductors). Three additional exercises targeted large lower-limb muscle groups (e.g., hip flexors, extensors, abductors, adductors; knee and ankle flexors and extensors). Lower-limb exercises were performed between upper-limb exercises to allow muscle relaxation. Each exercise lasted one minute, during which participants performed as many repetitions as possible. A two-minute rest period was provided between sets.

The program incorporated sports-based technical skills (Appendix IV - Examples of the exercise protocol) such as basketball (e.g., passing, catching, and throwing) and volleyball (e.g., passing and receiving). Fitness exercises included diagonal movements PNF techniques (Surburg & Schrader, 1997) using resistance bands and open-chain upper-limb exercises (e.g., shoulder flexion/extension or abduction/adduction with extended elbows) performed with 1 kg dumbbells.

Considering that the individual Rate of Perceived Exertion (RPE) should be within the range of 3 to 6, the progression and difficulty level were gradually enhanced. Progression was managed by increasing the distance of the passes and the resistance of the elastic bands according to each exercise. This approach ensured that the exercises remained challenging yet achievable, maximizing the benefits for participants without risking overexertion. Further details of the exercise protocol are provided in Table 3.3.

Table 3.3. Overview of the exercise protocol.

	Type of exercise	Duration	Body position	Difficulty level
1	Basketball chest pass	1 min	Standing	Distance of the pass
2	PNF 1st diagonal FP	1 min	Standing	Elastic band
3	Shoulders flexion/extension	1 min	Sitting	Hand dumbbells (1kg)
4	Adductors squeeze	1 min	Supine lying	Pilates ring
5	Basketball shoulder pass	1 min	Standing	Distance of the pass
6	PNF 1st diagonal EP	1 min	Standing	Elastic band
7	Shoulders abduction/adduction	1 min	Sitting	Hand dumbbells (1kg)
8	Hips Abduction	1 min	Supine lying	Pilates ring
9	Volleyball overhead pass	1 min	Standing	Distance of the pass
10	PNF 2nd diagonal FP	1 min	Standing	Elastic band
11	PNF 2nd diagonal EP	1 min	Standing	Elastic band
12	Squat	1 min	Standing	Balance pads

Table 3.3: PNF; Proprioceptive Neuromuscular Facilitation, FP; Flexion Pattern, EP; Extension Pattern, min; minute. Each session included three sets of nine different exercises which targeted large muscle groups of the upper limbs (i.e., 1–3 , 5–7, 9–11) and three exercises which targeted large muscle groups of the lower limbs (i.e., 4, 8, 12). Overall, for all participants the duration for each exercise was 1 minute or they were instructed to stop if they started to feel tired. The difficulty level for the sport activities tasks (i.e., 1, 5, 9) was maintained by changing the distance of the passes from the wall. The difficulty level for the exercises of the upper limbs (i.e., 2, 6,10, 11) was maintained by changing the resistance of elastic bands.

During the intervention phase, a physiotherapist at The Cyprus Institute of Neurology and Genetics conducted three clinical assessments (one every four weeks). These assessments included the completion of two questionnaires by each participant (Figure. 3.3).

Active Control Group

All exercise sessions and clinical assessments (see outcome measures) for the active control group were conducted at the Physiotherapy Unit of The Cyprus Institute of Neurology and Genetics. Participants in the active control group had one-on-one sessions with the same physiotherapist, whereas the experimental group participated in group training sessions.

Each session lasted approximately 60 minutes, and participants were required to complete at least 9 out of 12 sessions (75%) for their data to be included in the analysis (Akbar et al., 2020; Sokratous et al., 2023). Each intervention session consisted of three components:

1. A five-minute warm-up, including full-body range-of-motion exercises.
2. The main exercise program.
3. A five-minute cooldown, involving passive stretching of the muscle groups used during the session.

The participants in the active control group followed a conventional physiotherapy program that included strengthening major trunk muscles (Moon et al., 2013), resistance exercises for the upper limbs and treadmill exercise (Edwards & Pilutti, 2017).

Trunk Strengthening Exercises

The trunk-strengthening component consisted of 12 exercises (Appendix XI - Examples of conventional exercises) targeting the flexor (rectus abdominis) and extensor (erector spinae) muscles. During each exercise, participants held the final static position for three seconds and performed five to ten repetitions, depending on individual tolerance. A three-second pause was allowed between repetitions, with a maximum of one-minute rest between exercises. The difficulty level increased gradually by extending the hold time and increasing the number of repetitions, based on participants' tolerance.

Upper-Limb Strengthening Exercises

The upper-limb strengthening component included 12 exercises (Appendix XI - Examples of conventional exercises; Figures 3a, 3b, 3c) targeting the shoulder joint's flexor, extensor, internal rotator, and external rotator muscles, as well as the elbow joint's flexor and extensor muscles. Each exercise was performed for five to ten repetitions and

one to three sets, depending on individual tolerance. A three-second pause was allowed between repetitions, and a maximum of one-minute rest was provided between sets. The difficulty was gradually increased by either increasing the number of repetitions or the resistance of the elastic band, based on participants' capabilities.

Aerobic Exercises

To complete the session, all participants performed 10 – 15 minutes of treadmill walking at a pace of 1.5 – 2.5 km/h or cycling on a static cycle ergometer (MOTOmed Loop Parkinson) at 20 – 40 rpm (Appendix XI - Examples of conventional exercises; Figures 4 – 5). The intensity of these aerobic exercises was adjusted to individual tolerance.

During the intervention phase, the same physiotherapist who assessed the experimental group also conducted clinical assessments for the active control group. These assessments were performed every four weeks (three times in total) and included the completion of two questionnaires (see outcome measures) by each participant (Figure 3.3). All assessments took place at the Physiotherapy Unit of The Cyprus Institute of Neurology and Genetics.

3.1.4 Outcome Measures

Previous studies have indicated that information processing speed is the most common cognitive deficit pwPMS (Benedict et al., 2020; DeLuca et al., 2020) and it is correlated with manual dexterity (Low et al., 2017; Poirier, 1988). Therefore, this clinical trial examined whether a specific exercise protocol, based on in-phase bilateral upper limb exercises, led to greater improvement compared to the minimum exercise recommendation of the Cyprus General Health System. The primary outcome measured was information processing speed, assessed using the Symbol Digit Modalities Test. Secondary outcomes included manual dexterity, evaluated with the Purdue Pegboard Test (Gallus & Mathiowetz, 2003) changes in gait speed assessed by the Timed 25-Foot Walk Test (Motl, 2017), walking ability, balance, and coordination during gait evaluated by the Six Spot Step Test (Nieuwenhuis et al., 2006).

Since fatigue and QoL are key factors affecting pwMS, additional secondary outcome measures were included. These were the Modified Fatigue Impact Scale, a subjective questionnaire assessing the effects of fatigue (Fisk, Ritvo, et al., 1994) and the Medical

Outcomes Study Questionnaire Short Form 36 Health Survey, which is a tool for evaluating Health-Related QoL (Lins & Carvalho, 2016).

Cognitive processing and manual dexterity are closely intertwined, with each influencing the other. Several tasks requiring manual dexterity often engage cognitive processes, such as attention, memory, and problem-solving, suggesting a bidirectional relationship. This connection is particularly relevant to our study, as our exercise protocol includes bilateral upper limbs exercises. By targeting these motor skills, we anticipate an improvement in information processing speed, as the two are closely connected. Consequently, we have chosen information processing speed as our primary outcome measure, expecting it to be directly impacted by the effects of manual dexterity improvement, achieved through our intervention.

To ensure methodological consistency the same physiotherapist collected all data by performing the same methodological procedures in a quiet room, across all participants and across all time points, for both the experimental and active control group. To prevent any decline in performance due to participant fatigue, all assessments were conducted between 9 a.m. and 11 a.m. in the same sequence as it is described below.

3.1.5 Data Acquisition of Outcome Measures

Medical Outcomes Study Questionnaire Short Form 36 Health Survey.

This is a set of generic, coherent, and easily administered QoL subjective questionnaire (Ware et al., 1994). There are 11 questions in the specific questionnaire administered by an assessor, with 36 items in total covering eight domains scaled from 0 to 100. Higher values indicate better health status. The eight domains include: general health, vitality, physical function, role physical, bodily pain, role emotional, social functioning and mental health. Participants need between 5 to 10 minutes to complete the questionnaire.

Modified Fatigue Impact Scale

It is a subjective questionnaire describing the effects of fatigue during the past four weeks (Fisk, Ritvo, et al., 1994). The Modified Fatigue Impact Scale consists of 21 questions, which are rated from “0” (low rate) to “4” (high rate) and it is divided into three subscales (i.e., physical, cognitive, and psychosocial). The assessor records the total score of the

test as the final test result. A higher score indicates greater impact of fatigue in individuals' daily life.

Symbol Digit Modalities Test

It is a commonly used test in pwMS (Benedict et al., 2020) which measures processing speed as well as motor speed. We employed the oral form of the test, in which participants were provided with the test sheet with nine symbols, each paired with a number on top of the page, defined as the "key". For example, the symbol "O" is matched with the number "6", so the correct response would be "six". The rest of the page consists of a randomized, sequential variety of these symbols. Participants are asked to verbally respond with the number that corresponds with each symbol. During the test, participants are given two minutes to orally match symbols with digits as quickly as possible. The score is obtained by subtracting the number of errors from the number of items completed. To account for practice effects, we created six different forms of the Symbol Digit Modalities Test, as many as our assessment points, in which the order of the symbols and the numbers of the "key" were rearranged (Roar et al., 2016).

Purdue Pegboard Test

This is a standardized test of manual dexterity (Gallus & Mathiowetz, 2003). It consists of four subtests, performed on a board in which pins, washers and collars are placed by the participants into two parallel columns of holes, according to the subtest task. The first two subtests are unimanual tasks, which measure dexterity of the right and left hand, respectively. The third subtest is a synchronous bimanual task that requires simultaneous use of both hands to grasp and place the pins. In the fourth subtest, participants perform alternating movements of both hands to complete assemblies of different types of pegs. The score is calculated based on the number of pegs inserted in 30 seconds for the first three subtests, and in 1 minute for the fourth subtest.

Timed 25-Foot Walk

It is a quantitative assessment for mobility and lower limb function (Motl, 2017). Participants are directed to one end of a marked 25-foot path and are instructed to walk as quickly as possible. The time is recorded from the start and ends when participants reach the 25-foot mark. The same task is immediately run again by having the participants walk back the same distance. As our participants may be using assistive devices for

walking, they were instructed to use them for safety reasons. The final score was the mean score from the two completed trials.

Six Spot Step Test

It is a measure replicating a complex range of sensorimotor functions, such as lower limb strength, spasticity, coordination, as well as balance (Nieuwenhuis et al., 2006). It is a timed walking test that involves kicking over a number of targets placed along a 5-meter path. The specific test is cognitive demanding, that also includes coordination and dynamic balance. The final score was the mean time of the four runs (Callesen et al., 2019) (see page 68).

3.1.6 Analysis plan

Within-group analysis: For each outcome measure we calculated the mean values from each time point (separately for baseline and intervention). To investigate the effect of the exercise program on each of the outcome measures, separately for each group, we calculated the differences between phases' mean values. These differences reflect the degree of the intervention-elicited change on the clinical condition of all participants.

Between-groups analysis: To detect if there is a significant effect between the two study groups, comparisons were made between the difference of improvement between the two groups, for each outcome measure. The difference of improvement from each group was calculated by the difference between baseline and intervention mean values of each outcome variable.

Statistical analysis

A repeated measures ANOVA analysis was conducted to determine whether there were statistically significant differences in each outcome measure across the two study groups. After conducting the analysis, we identified variables with significant differences across groups. To further explore these differences, we performed post hoc pairwise comparisons using *t*-tests. To control for the risk of Type I errors due to multiple comparisons, a Bonferroni correction was applied to adjust the significance p – value.

Pearson correlation coefficient was used to determine the degree of correlation between the Symbol Digit Modalities Test and the Purdue Pegboard test. Bonferroni correction was used to determine which correlations were statistically significant. Data from the intervention phases of

both groups were used to define possible relationship between the two variables, to indicate the post-exercise correlation.

The null hypothesis was that "no improvement from the exercise program of the experimental group, compared to the active control group" for the outcome measure, thus participants' responses are independent from the condition (baseline vs. intervention) under which they were observed. The alternative hypothesis was that "the outcome measures of the experimental group would be affected by the specific intervention, when compared to the active control group". The null hypothesis was rejected if the *p* – value was smaller than the Bonferroni corrected *p* – value based on the actual number of tests that were performed (0.05/number of tests). All tests were two sided. Statistical analysis was performed using the statistical software JASP 0.19.1 (https://jasp-stats.org/).

Following our sampling plan, a total of twenty participants were enrolled and allocated to the experimental (n=10) and the active control (n=10) groups. All participants completed the exercise program without complaints or side effects. As described in detail below, results indicated that participants from the experimental group had on average greater improvement when compared with the controls on all outcome measures.

3.1.7 Results - Primary outcome measure

An increase in the total score in a given time point and the larger number of correct answers given from the participants, signified an improvement in information processing speed. The results from all participants during all time points are presented in Appendix XII - **Group comparison** – Individual results based on the outcome measure and group allocation; Table 1.

For information processing speed improvement, the ANOVA results (Appendix XIII - ANOVA analysis; Tables 1 – 3) revealed a significant improvement between the baseline and intervention phases for both the experimental and active control groups (Study Phases; $p < 0.001$) (Experimental Group: baseline mean score = 55.1 $\pm$ 13.8; intervention mean score = 61.3 $\pm$ 14.1, Active Control Group: baseline mean score = 55 $\pm$ 13.4; intervention mean score = 57.2 $\pm$ 13.2). Interaction analysis showed a significant interaction between study phases and group (Study Phases ✻ Group; $p < 0.001$) (Appendix XII - ANOVA analysis; Table 1), indicating that the experimental group demonstrated more pronounced improvement, as reflected in their mean scores.

Importantly, the baseline characteristics of the experimental and active control groups, based on their mean scores, showed no significant differences (Figure 3.5). This suggests that both groups started from comparable baselines, minimizing the potential influence of initial differences on the outcomes.

The Post Hoc analysis based on the Bonferroni correction indicated significant improvement between the baseline and intervention phases for both study groups ($p < 0.001$) (Appendix XIII - ANOVA analysis; Table 2 – 3). A *t*-test was conducted in which significant improvement difference (Experimental group improvement = 6.2 ± 1.4, Active control group improvement = 2.2 ± 0.6) was detected between the experimental and active control groups; $t\ (18) = 8.6$, $p < 0.05$ (Appendix XIII - ANOVA analysis; Table 4).

Figure 3.5. Symbol Digit Modalities Test – Group comparison.

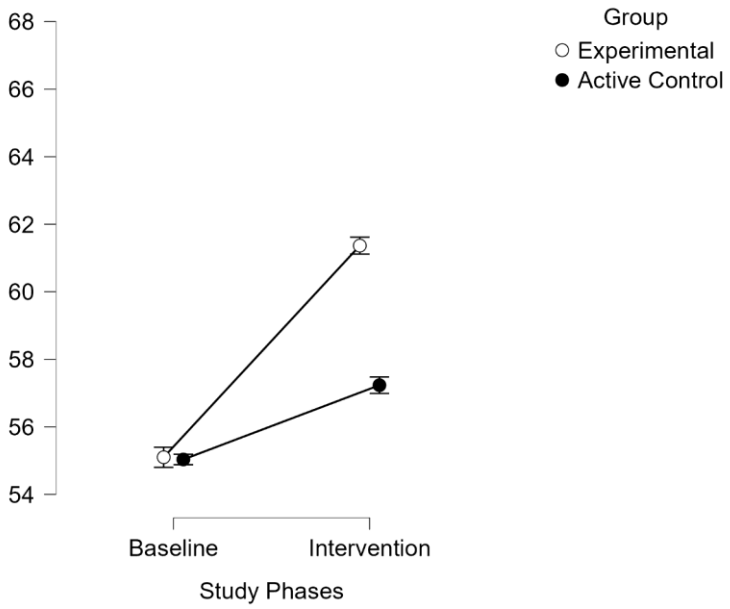


Figure 3.5: Both groups started from comparable baselines, minimizing the potential influence of initial differences on the outcomes. The experimental group demonstrated a more pronounced improvement.

Focused on the primary outcome, the Symbol Digit Modalities Test, with an estimated effect size of partial $\eta^2 = 0.807$, a significance level of $\alpha = 0.05$, and a sample size of 20 participants, a post hoc analysis indicated that the study had 100% statistical power to detect associations ($1 - \beta = 1$). The power analysis was conducted using G*Power (Figure 3.6) (Erdfelder & Buchner, 1996).

Figure 3.6. Power analysis procedure via G*Power.

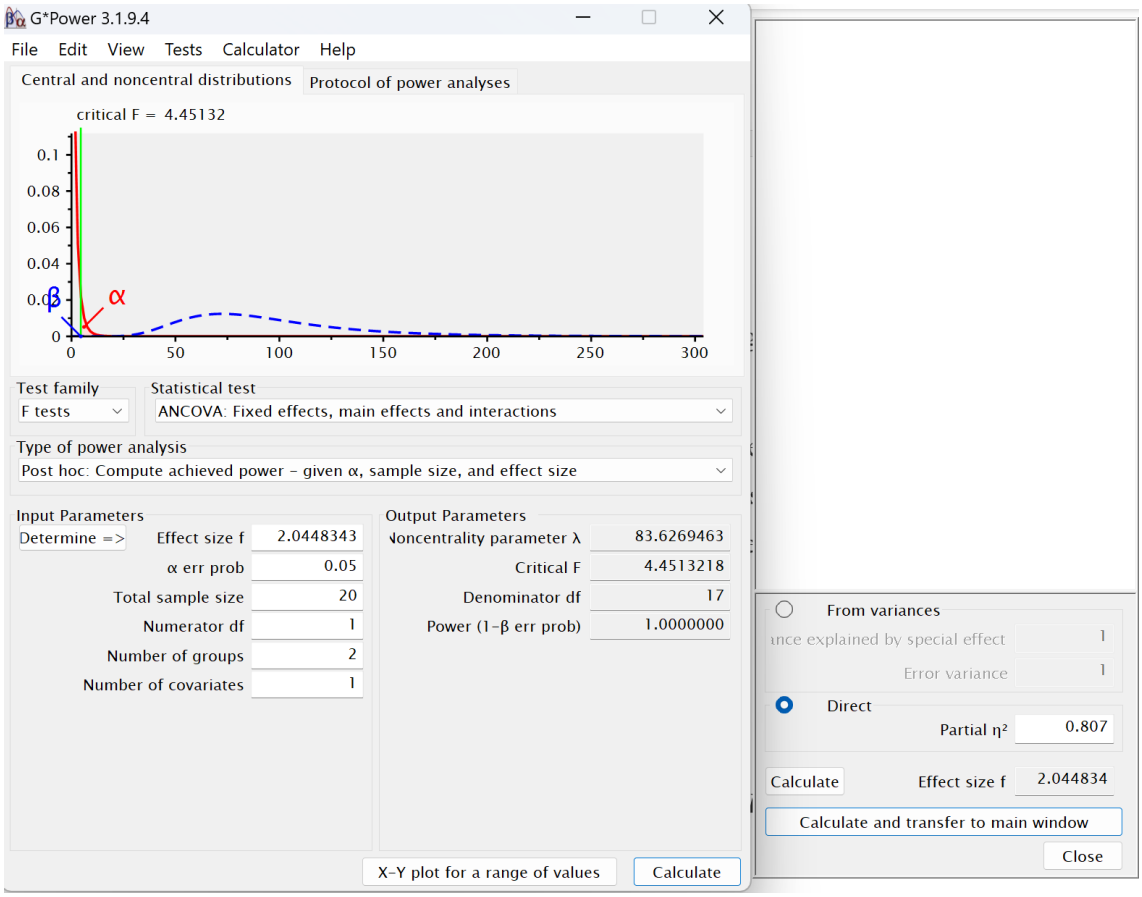


Figure 3.6: G*Power is a statistical software program used to calculate statistical power, effect sizes, and sample size requirements for various statistical tests. A Post Hoc analysis conducted using G*Power indicated that the study achieved 100% power for the primary outcome measure (i.e., Symbol Digit Modalities Test), with 20 participants divided into two independent groups.

3.1.8 Results - Secondary outcome measures

Medical Outcomes Study Questionnaire Short Form 36 Health Survey

Statistical analysis was conducted to determine whether there were statistically significant differences in Medical Outcomes Study Questionnaire Short Form 36 Health Survey score across the two study groups (Appendix XIII - ANOVA analysis; Tables 38 – 40). A higher score indicated improvement of the participants' QoL. During the baseline phase all participants completed the specific questionnaire only at the last assessment point, prior to the intervention phase. However, during the intervention phase all participants completed the questionnaire three times (i.e., once per four weeks). The results from all

participants during all time points are presented in Appendix XII - Group comparison– Individual results based on the outcome measure and group allocation; Table 2.

To detect improvement in QoL an ANOVA analysis was performed (Appendix XIII - ANOVA analysis; Tables 5 – 8). The results indicated a significant improvement between the baseline and intervention phases only for the experimental (Study Phases; $p = 0.05$) (Experimental Group: baseline mean score = 88.8 $\pm$ 6.1; intervention mean score = 95.6 $\pm$ 55.5, Active Control Group: baseline mean score = 85.7 $\pm$ 8.3; intervention mean score = 85.6 $\pm$ 6.7). Interaction analysis showed a significant interaction between study phases and group (Study Phases ✻ Group; $p < 0.001$) (Appendix XIII - ANOVA analysis; Table 5), indicating that the experimental group demonstrated more pronounced improvement, as reflected in their mean scores. Importantly, the baseline characteristics of the experimental and active control groups, based on their mean scores, showed no significant differences (Figure 3.7). This suggests that both groups started from comparable baselines, minimizing the potential influence of initial differences on the outcomes.

The Post Hoc analysis based on the Bonferroni correction indicated statistically significant difference in the experimental group between the baseline and all the intervention assessment points (I1; $p < 0.001$, I2; $p < 0.001$, I3; $p = 0.013$), whereas this did not observe in the active control group (Appendix XIII - ANOVA analysis; Tables 6 – 7). A *t*-test was conducted in which statistically significant improvement difference (Experimental group improvement = 6.8 $\pm$ 2.3, Active control group improvement = -0.06 $\pm$ 2.9) was detected between the experimental and active control groups; $t\ (18) = 7.03$, $p < 0.05$ (Appendix XIII - ANOVA analysis; Table 8).

Figure 3.7. Medical Outcomes Study Questionnaire Short Form 36 Health Survey – Group comparison.

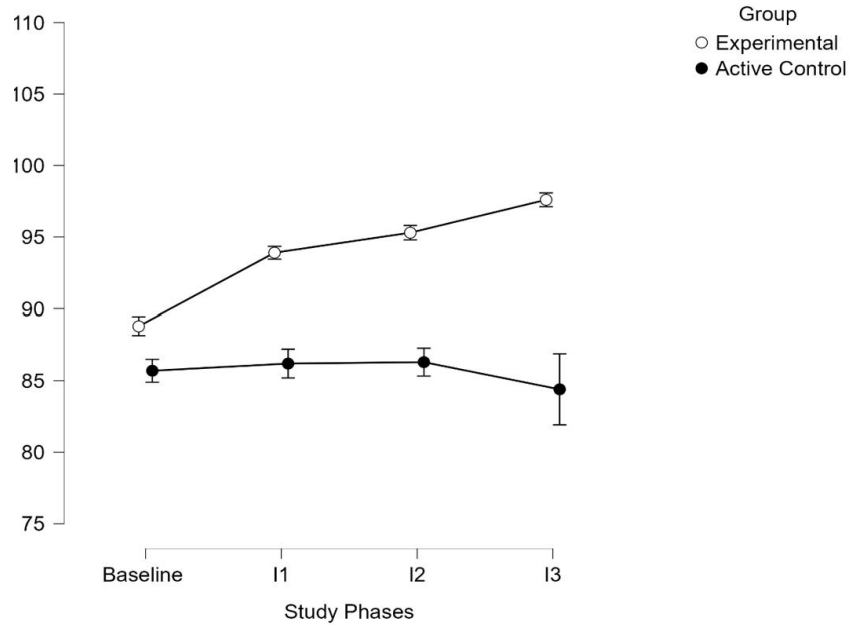


Figure 3.7: I; Intervention. An improvement was observed between the baseline and all intervention points for the experimental group. Moreover, a statistically significant improvement was observed in the experimental group compared to the active control group.

Modified Fatigue Impact Scale

The analysis was conducted to determine whether there were statistically significant differences in Modified Fatigue Impact Scale score across the two study groups (Appendix XIII - ANOVA analysis; Tables 9 – 12). A decrease score in Modified Fatigue Impact Scale indicated an improvement of the participants' fatigue level. During the baseline phase all participants completed the specific questionnaire only at the last assessment point, prior to the intervention phase. During the intervention phase all participants completed the questionnaire three times (i.e., once per four weeks). The results from all participants during all time points are presented in Appendix XII - Group comparison – Individual results based on the outcome measure and group allocation; Table 3.

For the improvement in fatigue level, the results from the ANOVA analysis (Appendix XIII - ANOVA analysis; Tables 9 – 12) indicated a significant improvement between the baseline and intervention phases for both the experimental and active control groups (Study Phases; $p < 0.001$) (Experimental Group: baseline mean score = 34 $\pm$ 14.8; intervention mean score = 26.7 $\pm$ 15, Active Control Group: baseline mean score = 44.8

± 19.9; intervention mean score = 45.3 ± 20.3). Interaction analysis indicated a significant interaction (Study Phases ✻ Group; $p < 0.001$) (Appendix XIII - ANOVA analysis; Table 9) between study phases and group. Notably, the baseline characteristics of the experimental and active control groups, as indicated by their mean scores, showed no significant differences (Figure 3.8). This suggests that both groups started from comparable baselines, minimizing the potential influence of initial differences on the outcomes.

The Post Hoc analysis and Bonferroni correction indicated statistically significant difference in the experimental group between the baseline and all the intervention assessment points (I1; $p = 0.004$, I2; $p < 0.001$, I3; $p < 0.001$), whereas this did not observe in the active control group (Appendix XIII - ANOVA analysis; Tables 10 – 11). A *t*-test was conducted in which statistical significant improvement difference (Experimental group improvement = 10.6 ± 7.9, Active control group improvement = -0.3 ± 1.6) was detected between the experimental and active control groups; $t(18) = 4.1$, $p < 0.05$ (Appendix XIII - ANOVA analysis; Table 12).

Figure 3.8. Modified Fatigue Impact Scale – Group comparison.

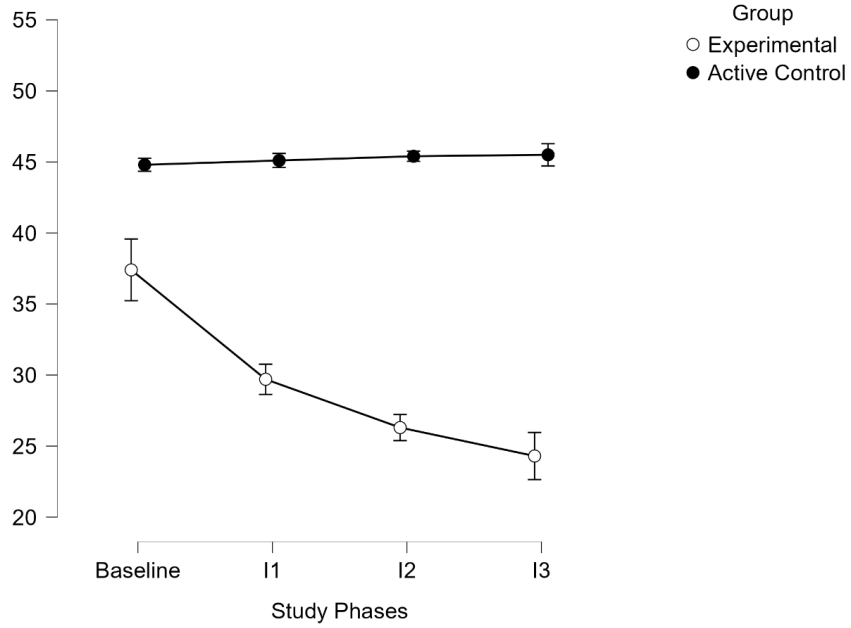


Figure 3.8: Improvement of the fatigue level, from baseline to intervention, was observed only in the experimental group. However, a progressive decline of the fatigue was observed in the active control group.

Purdue Pegboard Test

Statistical analysis was conducted to determine whether there were statistically significant differences in the four subtests score across the two study groups (Appendix XIII - ANOVA analysis; Tables 13 – 18). Higher score in the Purdue Pegboard Test, indicates improvement of the participants' manual dexterity (i.e., unimanual dominant, unimanual non-dominant, bimanual, assembly). During both baseline and intervention phases, all participants performed the specific test three times in each phase (see Figure 3.3). The results from all participants during all time points are presented in Appendix XII - Individual results based on the outcome measure and group allocation; Tables 4 – 8 (*Unimanual Dominant hand subtest* Experimental Group: baseline mean score = 12.2 $\pm$ 2.3; intervention mean score = 14.2 $\pm$ 2.3, Active Control Group: baseline mean score = 10.7 $\pm$ 1.8; intervention mean score = 10.5 $\pm$ 1.3. *Unimanual Non-Dominant hand subtest* Experimental Group: baseline mean score = 10.3 $\pm$ 2, intervention mean score = 11.8 $\pm$ 2.2, Active Control Group: baseline mean score = 9.1 $\pm$ 2.3; intervention mean score = 8.8 $\pm$ 1. *Bimanual hand subtest* Experimental Group: baseline mean score = 9.4 $\pm$ 1.3; intervention mean score = 11.1 $\pm$ 1.7, Active Control Group: baseline mean score = 8.6 $\pm$ 1.6; intervention mean score = 8.9 $\pm$ 1.1. *Assembly hand subtest* Experimental Group: baseline mean score = 17.6 $\pm$ 4.5; intervention mean score = 22.3 $\pm$ 5.4, Active Control Group: baseline mean score = 14.7 $\pm$ 3.5; intervention mean score = 15.1 $\pm$ 3).

For the of improvement in manual dexterity, the results from the ANOVA analysis (Appendix XIII - ANOVA analysis; Table 13) indicated significant differences; a) between baseline and intervention phases for both experimental and active control groups (Study Phases; $p < 0.001$) and b) between subtests (Subtests; $p < 0.001$). Interaction analysis indicated a significant interaction a) between study phases and group (Study Phases ✻ Group; $p < 0.001$), b) between subtests and group (Subtests ✻ Group; $p < 0.007$) and c) between study phases, subtests and group (Study Phases ✻ Subtests ✻ Group; $p < 0.001$) (Appendix XIII - ANOVA analysis; Table 13). Importantly, the baseline characteristics of the experimental and active control groups, based on mean scores, showed no statistically significant differences ($p = 1$ for all subtests) (Appendix XIII - ANOVA analysis; Table 16) (Figures 3.9 – 3.12). This confirms that both groups started from comparable baselines, reducing the likelihood that initial differences influenced the observed outcomes.

After conducting a Post Hoc analysis based on the Bonferroni correction, the difference between baseline and intervention which was observed in the experimental group, was statistically significant ($p < 0.001$) (Appendix XIII - ANOVA analysis; Table 14). Also, the differences observed between the four subtests were statistically significant, except the one between Unimanual Non-Dominant and Bimanual ($p < 0.27$) (Appendix XIII - ANOVA analysis; Table 15).

From the within group statistical analysis, differences were observed in the mean scores of baseline phases between the four subtests, for both study groups. In the experimental group these differences were statistically significant between a) Unimanual Dominant and Non-Dominant subtests ($p < 0.005$), b) Unimanual Dominant and Bimanual subtests ($p < 0.002$), c) Unimanual Dominant and Assembly tests ($p < 0.001$), d) Unimanual No-Dominant and Assembly subtests ($p < 0.001$) and d) Bimanual and Assembly subtests ($p < 0.001$). The difference between Unimanual No-Dominant and Bimanual subtests ($p = 1$) was not statistically significant (Appendix XIII - ANOVA analysis; Table 16). On the other hand, in the active control group the differences which were observed were statistically significant between a) Unimanual Dominant and Non-Dominant subtests ($p = 0.04$), b) Unimanual Dominant and Bimanual subtests ($p = 0.03$), c) Unimanual Dominant and Assembly tests ($p = 0.01$), d) Unimanual No-Dominant and Assembly subtests ($p = 0.002$) and d) Bimanual and Assembly subtests ($p = 0.002$). The difference between Unimanual No-Dominant and Bimanual subtests ($p = 1$) was not statistically significant (Appendix IX - ANOVA analysis; Table 16).

Throughout the within group statistical analysis, the differences observed in the experimental group between baseline and intervention phases, were statistically significant for the Unimanual Dominant ($p < 0.001$), Bimanual ($p < 0.004$), Assembly ($p < 0.001$) subtests, whereas this was not demonstrated in the Unimanual Non-Dominant subtest ($p < 0.6$) in the experimental group and to all subtests in the active control group ($p = 1$) (Appendix XIII - ANOVA analysis; Table 16).

Based on the between groups statistical analysis, between baseline phases of the two study groups, no statistically significant difference was observed to any of the subtests ($p < 0.05$) (Appendix XIII - ANOVA analysis; Table 16). This suggests that both groups started from comparable baselines, minimizing the potential influence of initial differences on the outcomes.

Since differences were observed between the rate of improvement in each test and between both study groups, an ANOVA analysis was performed to detect if there were statistically significant differences. During the analysis between the subtests and the groups, statistically significant improvement was observed within the four subtests (Purdue Pegboard Subtests; $p < 0.001$). Interaction analysis indicated a significant interaction a) between study phases and group (Purdue Pegboard Subtests; ✻ Group; $p < 0.001$) (Appendix XIII - ANOVA analysis; Table 17).

According to the within group statistical analysis, the differences in the improvement observed in the experimental group between the subtests, were statistically significant only between a) Unimanual Dominant and Assembly subtests ($p = 0.006$), b) Unimanual Non-Dominant and Assembly subtests ($p = 0.004$) and c) Bimanual and Assembly subtests ($p < 0.001$). This was not detected between a) Unimanual Dominant and Non-Dominant subtest ($p = 1$), b) Unimanual Dominant and Bimanual subtests ($p = 1$) and c) Unimanual Non-Dominant and Bimanual subtests ($p = 1$) in the experimental group and to all subtests in the active control group ($p = 1$) (Appendix XIII - ANOVA analysis; Table 18).

Figure 3.9. Unimanual Dominant Subtest – Group comparison.

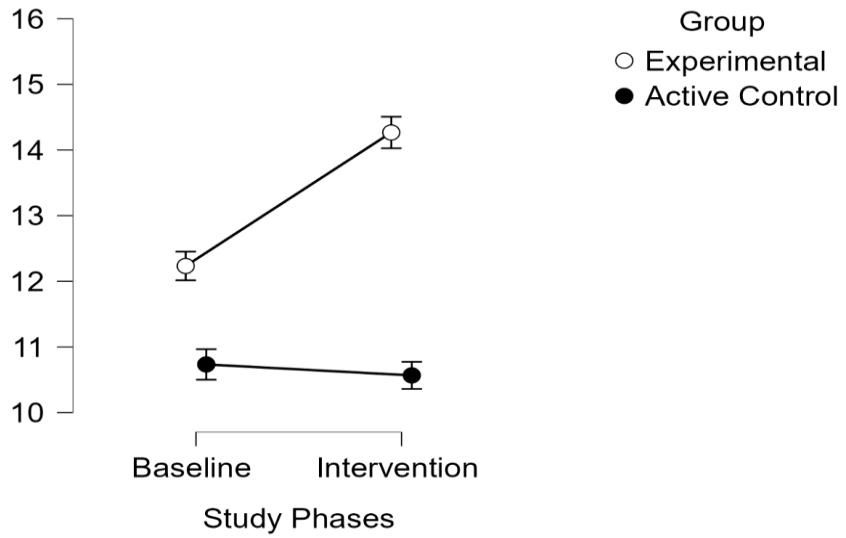


Figure 3.9: A statistically significant improvement was observed between baseline and intervention of the experimental group ($p < 0.001$), whereas this was not detected in the active control group, as indicated form the statistical analysis.

Figure 3.10. Unimanual Non-Dominant Subtest – Group comparison.

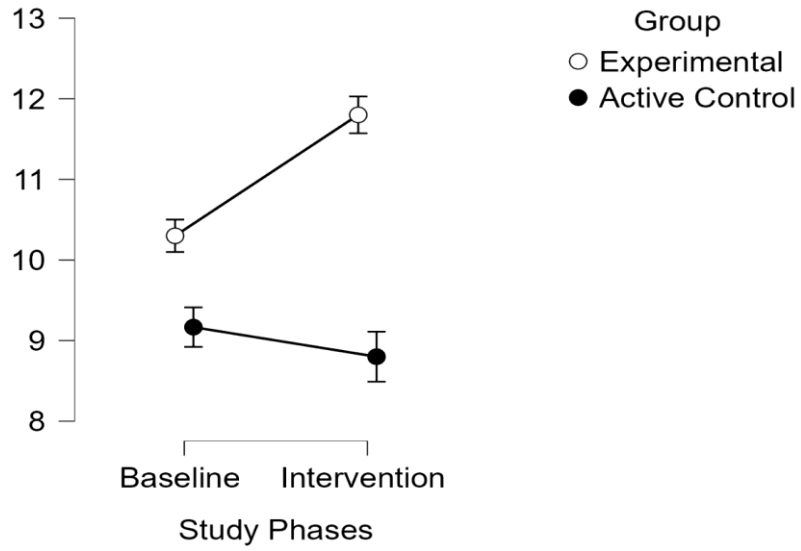


Figure 3.10: A statistically significant improvement was observed between baseline and intervention of the experimental group ($p < 0.001$), whereas this was not detected in the active control group, as indicated form the statistical analysis.

Figure 3.11. Bimanual Subtest – Group comparison.

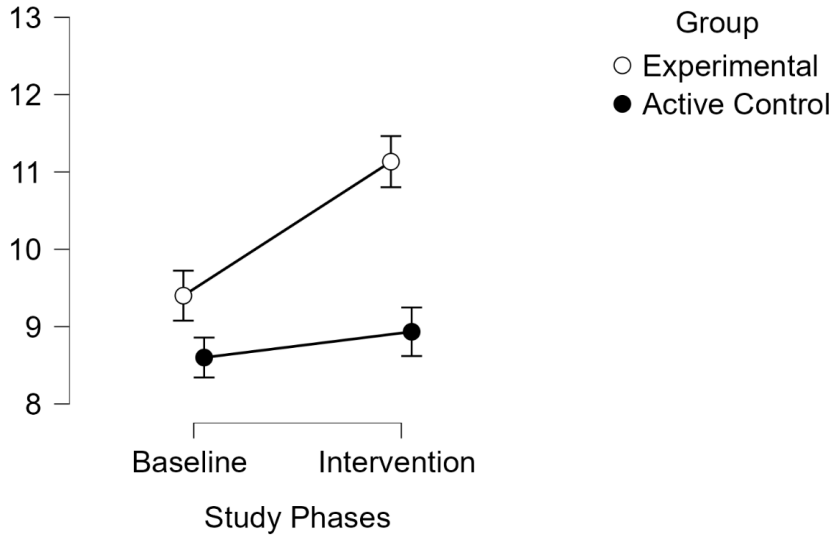


Figure 3.11: A statistically significant improvement was observed between baseline and intervention of the experimental group ($p < 0.001$), whereas this was not detected in the active control group, as indicated form the statistical analysis.

Figure 3.12. Assembly Subtest – Group comparison.

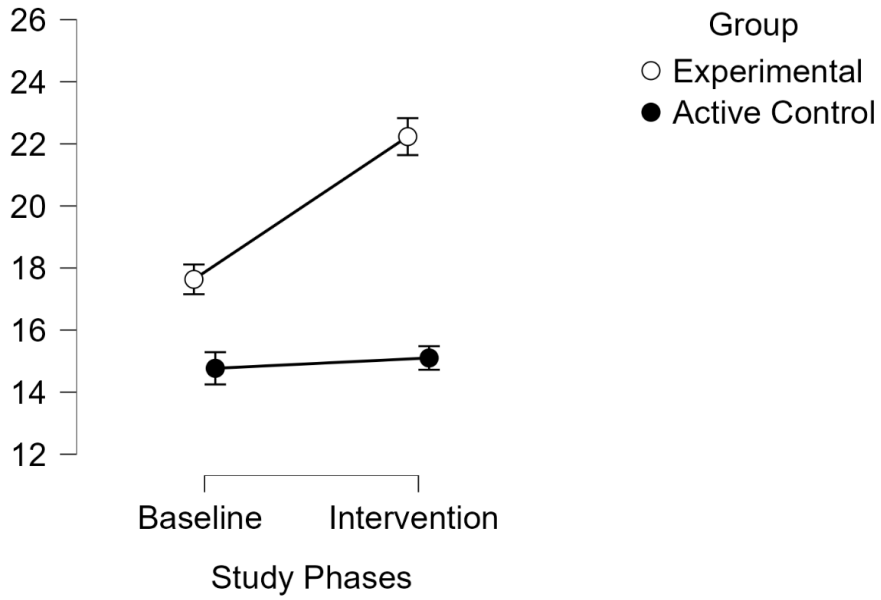


Figure 3.12: A statistically significant improvement was observed between baseline and intervention of the experimental group ($p < 0.001$), whereas this was not detected in the active control group, as indicated form the statistical analysis.

When comparing the improvement within the experimental group to the active control group's intervention results, significant improvement was noted in the Unimanual Dominant ($p = 0.004$) and Assembly ($p < 0.001$) subtests, while no significant improvement was found for the Unimanual Non-dominant ($p = 0.32$) or Bimanual ($p = 0.16$) subtests (Appendix XIII - ANOVA analysis; Table 18). The greatest improvement in the experimental group was observed in the Assembly subtest (4.6 pegs), compared to the Unimanual Dominant (2 pegs), Unimanual Non-dominant (1.5 pegs), and Bimanual (1.7 pegs) subtests (Figure 3.13) (Appendix XII - Group comparison – Individual results based on outcome measure and group allocation; Table 8).

Figure 3.13. Purdue Pegboard Subtests improvement – between groups.

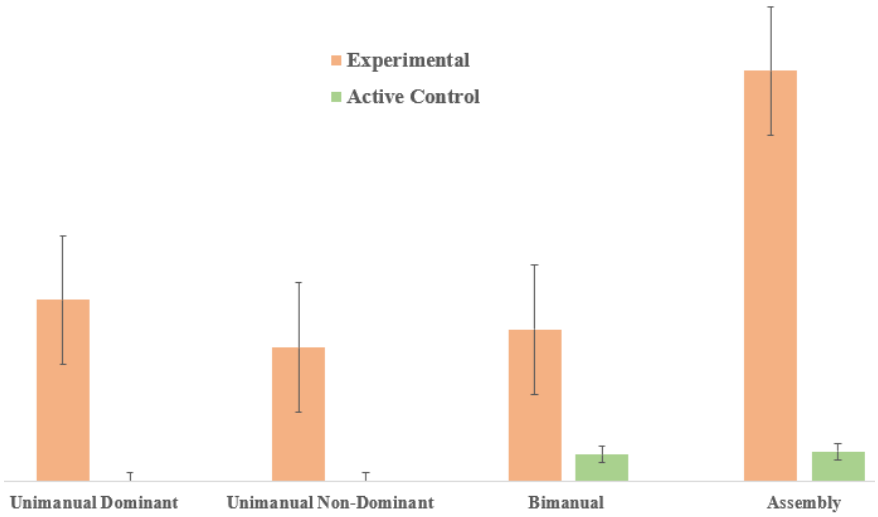


Figure 3.13*:* The results for the Unimanual Dominant and Non-Dominant subtests in the active control group were negative, so they were considered as zero (no improvement). Improvement was primarily observed in the experimental group. In Assembly subtest (4.6 points) was the greatest statistically significant improvement, compared to the other subtests: Unimanual Dominant (2 points), Unimanual Non-Dominant (1.5 points), and Bimanual (1.7 points).

Timed 25-Foot Walk

Statistical analysis was conducted to determine whether there were statistically significant differences in Timed 25-Foot Walk test across the two study groups. A decrease of the time needed to perform the test, signified improvement of the participants' gait. The results from all participants during all time points are presented in Appendix XII - Group comparison – Individual results based on the outcome measure and group allocation; Table 9.

For the detection of improvement in gait, the results from the ANOVA (Appendix XIII - ANOVA analysis; Tables 19 – 22) indicated a statistical significant improvement between the baseline and intervention phases only both the experimental group (Study Phases; $p < 0.001$) (Experimental Group: baseline mean score = 9s $\pm$ 2.3; intervention mean score = 6.7s $\pm$ 2, Active Control Group: baseline mean score = 9.4s $\pm$ 2.7; intervention mean score = 9.5s $\pm$ 2.8). Interaction analysis indicated a significant interaction (Study Phases ✻ Group; $p < 0.001$) (Appendix XIII - ANOVA analysis; Table 19) between study phases and group. Notably, the baseline characteristics of the experimental and active control groups, as indicated by their mean scores, showed no

statistical significant differences after the Post Hoc analysis based on the Bonferroni correction ($p = 1$) (Appendix XIII - ANOVA analysis; Table 21). This suggests that both groups started from comparable baselines, minimizing the potential influence of initial differences on the outcomes (Figure 3.14).

The Post Hoc analysis based on the Bonferroni correction, demonstrated statistically significant improvement between baseline and intervention of the experimental group ($p < 0.001$), whereas this was not detected in the active control group ($p = 1$) (Appendix XIII - ANOVA analysis; Table 21). A *t*-test was conducted in which significant statistical improvement difference (Experimental group improvement = 2.2 $\pm$ 0.7, Active control group improvement = -0.05s $\pm$ 0.2) was detected between the experimental and active control groups; $t(18) = 8.6$, $p < 0.05$ (Appendix XIII - ANOVA analysis; Table 22).

Figure 3.14. Timed 25-Foot Walk Test – Group comparison.

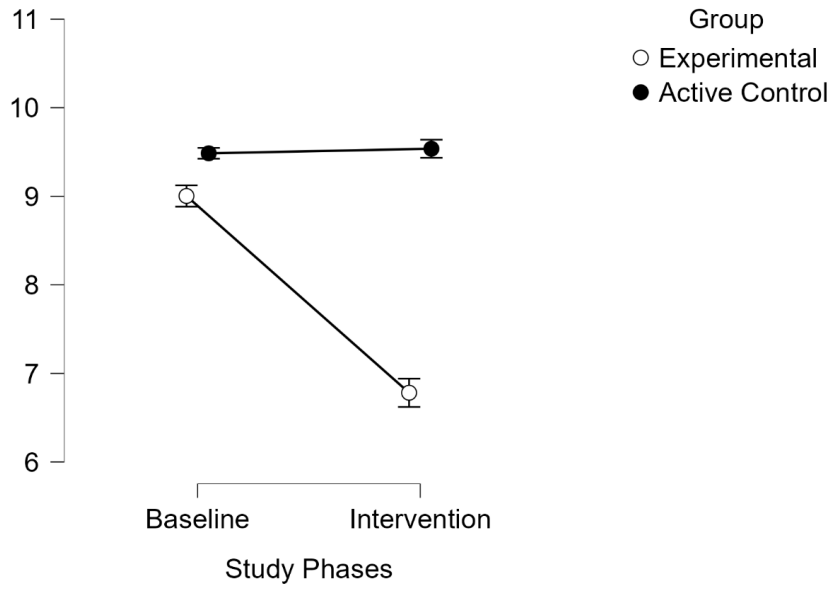


Figure 3.14: A statistically significant improvement was observed between baseline and intervention of the experimental group ($p < 0.001$), whereas this was not detected in the active control group ($p = 1$), as indicated form the statistical analysis.

Six Spot Step Test

Statistical analysis was conducted to determine whether there were statistically significant differences in the Six Spot Step Test across the two study groups. A decrease of the time needed to perform the test, signified improvement of the participants' gait. The results from all participants during all time points are presented in Appendix XII - Group

comparison – Individual results based on the outcome measure and group allocation; Table 10.

For the detection of improvement in gait, the results from the ANOVA (Appendix XIII - ANOVA analysis; Tables 23 – 26) indicated a statistical significant improvement between the baseline and intervention phases only for the experimental group (Study Phases; $p < 0.001$) (Experimental Group: baseline mean score = 13.3s $\pm$ 8.3; intervention mean score = 11s $\pm$ 6.6, Active Control Group: baseline mean score = 16.4s $\pm$ 8.7; intervention mean score = 16.5s $\pm$ 8.9). Interaction analysis indicated a significant interaction (Study Phases ✻ Group; $p < 0.001$) (Appendix XIII - ANOVA analysis; Table 23) between study phases and group. Notably, the baseline characteristics of the experimental and active control groups, as indicated by their mean scores, showed no statistical significant differences after the Post Hoc analysis based on the Bonferroni correction ($p = 1$) (Appendix XIII - ANOVA analysis; Table 25). This suggests that both groups started from comparable baselines, minimizing the potential influence of initial differences on the outcomes (Figure 3.15).

The Post Hoc analysis based on the Bonferroni correction, demonstrated statistical significant improvement between baseline and intervention of the experimental group ($p < 0.001$), whereas this was not detected in the active control group ($p = 1$) (Appendix XIII - ANOVA analysis; Table 25). A *t*-test was conducted in which statistical significant improvement difference (Experimental group improvement = 2.3 $\pm$ 1.7, Active control group improvement = -0.1 $\pm$ 0.2) was detected between the experimental and active control groups; $t(9) = 4$, $p < 0.05$ (Appendix XIII - ANOVA analysis; Table 26).

Figure 3.15. Six Spot Step Test – Group comparison.

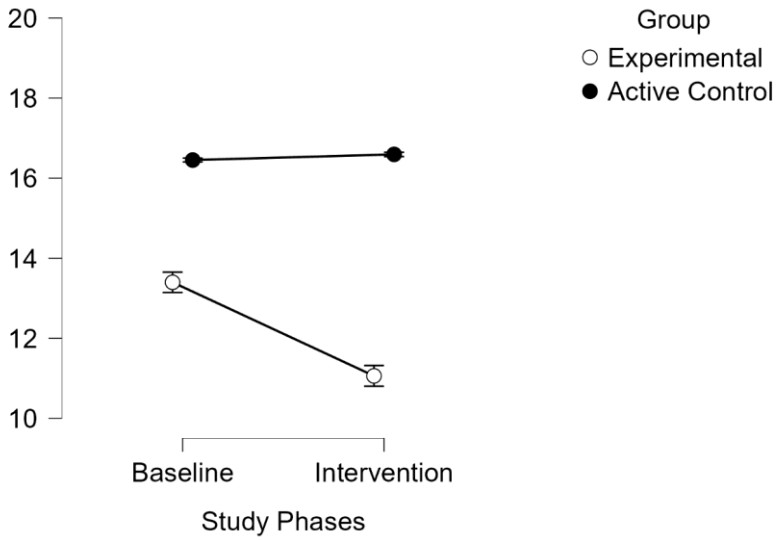


Figure 3.15*:* A statistically significant improvement was observed between baseline and intervention of the experimental group ($p < 0.001$), whereas this was not detected in the active control group ($p = 1$), as indicated form the statistical analysis.

Correlation Symbol Digit Modalities Test and Purdue Pegboard Test

Pearson's correlation coefficient (Pearson's r) was calculated using the mean score from the intervention phase to examine the relationship between information processing speed and manual dexterity. The analysis included the Symbol Digit Modalities Test and the sub-tests of the Purdue Pegboard test. In the experimental group (Tables 3.4 and 4.5) non-significant moderate correlation was observed between the Symbol Digit Modalities Test and the unimanual dominant hand dexterity (r = 0.6, $p = 0.07$), the unimanual non-dominant hand dexterity (r = 0.6, $p = 0.09$) and the bimanual dexterity (r = 0.5, $p = 0.1$). However, significant moderate correlation was observed between the Symbol Digit Modalities Test and the assembly dexterity (r = 0.7, $p = 0.02$). On the other hand, in the active control group (see Tables 3.4 and 3.5) non-significant weak correlation was found between the Symbol Digit Modalities Test and the Purdue pegboard sub-tests (unimanual dominant hand: r = 0.3, $p = 0.4$; unimanual non-dominant hand: r = 0.5, $p = 0.1$; bimanual: r = 0.5, $p = 0.1$; assembly: r = 0.2, $p = 0.6$). Nevertheless, the highest significant correlation was found in the experimental group, between two couplings of Purdue pegboard sub-tests, the unimanual non-dominant hand - bimanual (r = 0.9, $p = 0.001$) and the unimanual non-dominant hand - assembly (r = 0.9, $p = 0.001$).

Following Bonferroni correction for five tests, statistically significant *p* – value is equal or less than 0.01 (*p* = 0.05 / number of tests = 5).

Table 3.4. Experimental group correlation between Symbol Digit Modalities Test and Purdue Pegboard Test.

	SDMT	**Dominant hand**	**Non-dominant hand**	**Bimanual**	**Assembly**
SDMT					
Dominant hand	r = 0.6 *p* = 0.07				
Non-dominant hand	r = 0.6 *p* = 0.09	r = 0.8 *p* = 0.006 *			
Bimanual	r = 0.5 *p* = 0.1	r = 0.8 *p* = 0.01 *	r = 0.9 *p* = 0.001 *		
Assembly	r = 0.7 *p* = 0.02	r = 0.8 *p* = 0.007 *	r = 0.9 *p* = 0.001 *	r = 0.8 *p* = 0.006 *	

Table 3.4: SDMT; Symbol Digit Modalities Test, r; Pearson correlation coefficient, p; p – value, *; Statistically significant results after Bonferroni correction.

Table 3.5. Active control group correlation between Symbol Digit Modalities Test and Purdue Pegboard Test.

	SDMT	Dominant hand	Non-dominant hand	Bimanual	Assembly
SDMT					
Dominant hand	r = 0.3 *p* = 0.4				
Non-dominant hand	r = 0.5 *p* = 0.1	r = 0.7 *p* = 0.02			
Bimanual	r = 0.5 *p* = 0.1	r = 0.6 *p* = 0.05	r = 0.5 *p* = 0.1		
Assembly	r = 0.2 *p* = 0.6	r = 0.7 *p* = 0.04	r = 0.3 *p* = 0.3	r = 0.2 *p* = 0.5	

Table 3.5: SDMT; Symbol Digit Modalities Test, r; Pearson correlation coefficient, p; p – value. Result showed no statistically significant improvement after Bonferroni correction.

3.2 Chapter Summary

The study aimed to investigate the impact of an in-phase bilateral upper limb exercise program on information processing speed and manual dexterity in pwPMS. Our primary hypothesis—that a 12-week exercise program focusing on in-phase bilateral upper limb movements could improve information processing speed compared to conventional exercises does have an effect on that—was supported by the results. The experimental group demonstrated improved information processing speed following the intervention.

However, our secondary hypothesis—that improvements in information processing speed would also lead to enhancements in manual dexterity within this clinical cohort—was not supported by the data. Although a moderate correlation was observed in the experimental

group, it was not statistically significant. The exercise protocol led to statistically significant improvements across all outcome measures in the experimental group compared to the active control group. Beyond examining the relationship between cognitive functions and manual dexterity, the specific exercise protocol demonstrated a strong, significant correlation between unimanual dexterity of the non-dominant hand and both bimanual dexterity and assembly skills. These findings support the hypothesis that bilateral exercises enhance communication and coordination between the upper limbs by activating both brain hemispheres (Asemi et al., 2015; Grefkes et al., 2008; Rudisch et al., 2020; Swinnen & Wenderoth, 2004). This activation improves the brain's ability to control both hands simultaneously, which is crucial for complex manual tasks, such as assembly. The exercise improved balance, gait, fatigue and QoL, highlighting in-phase bilateral exercises' potential for cognitive and motor rehabilitation in pwPMS.

CHAPTER 4

General Discussion and Conclusion

4.1 Discussion

As far as we know, people with Multiple Sclerosis (pwMS) exhibit a broad spectrum of clinical symptoms, including motor and cognitive impairments, which vary based on disease progression. In people with Relapsing-Remitting Multiple Sclerosis (pwRRMS), rehabilitation and physiotherapeutic interventions primarily target motor function by promoting corticospinal plasticity, aiming to restore movement and reduce disability. Conversely, in Progressive Multiple Sclerosis (PMS) (i.e., Primary Progressive Multiple Sclerosis / Secondary Progressive Multiple Sclerosis), both cognitive and motor dysfunctions significantly influence rehabilitation strategies, as cognitive decline profoundly affects daily functioning and quality of life (QoL). Consequently, physiotherapeutic approaches in PMS integrate motor rehabilitation alongside cognitive training to address the broader range of neurological deficits associated with disease progression.

This thesis aimed to investigate whether exercises based on in-phase bilateral upper limb movements could enhance corticospinal plasticity while improving both cognitive functions and motor skills. To address this question, two clinical trials were conducted.

The first clinical trial (Chapter 2.3) explored the effects of in-phase bilateral upper limb exercises on corticospinal plasticity and clinical outcomes in pwRRMS. A pilot single-case study (n=1) was initially performed to assess the feasibility and safety of the proposed methodology, followed by a concurrent multiple-baseline design study involving five pwRRMS (n=5), all undergoing the same intervention protocol. Given the prevalence of cognitive impairments in pwMS, a second clinical trial (Chapter 3) was designed to assess the impact of in-phase bilateral upper limb exercises on information processing speed, alongside with improvement on manual dexterity and other clinical measures. This study included 20 pwPMS in total (n=20), who were alocated into an experimental group (n=10) performing in-phase bilateral upper limb exercises and an active control group (n=10) following a conventional physiotherapy program.

The results demonstrated that the proposed in-phase bilateral upper limb exercise protocols significantly influenced resting motor threshold (rMT) in pwRRMS and led to notable improvements in information processing speed and other clinical measures in both pwRRMS and pwPMS. Building on these findings, this chapter synthesizes and

discusses the results of the above-mentioned studies by, highlighting key limitations, and exploring their translational potential while outlining future research directions.

4.1.1 The Effects of In-Phase Bilateral Exercises in Relapsing-Remitting Multiple Sclerosis

A specific exercise protocol (see page 146) was designed to investigate the influence of an in-phase bilateral upper limb exercise regimen on corticospinal plasticity, with a particular focus on its effects on central motor conduction time (CMCT). Additionally, the study aimed to evaluate the secondary impact of the protocol on clinical outcomes in pwRRMS.

Contrary to expectations, the exercise protocol did not result in significant changes in CMCT. However, it had a notable effect on rMT, a key indicator of cortical excitability and plasticity (Caramia et al., 2004; Hallett, 2007) and a significant impact on motor function and cognitive processing. This finding suggests that while the exercises did not enhance neural conduction speed, they may have contributed to increased neural excitability and improved the QoL of individuals.

Although the first hypothesis predicted that the exercise protocol would enhance corticospinal plasticity through a reduction in CMCT, the results only partially supported this expectation. This underscores the complexity of modulating corticospinal plasticity and the need for further research to clarify the mechanisms by which exercise influences neurophysiological parameters in this population. Importantly, the study suggests that targeted exercise interventions can yield meaningful clinical improvements even in the absence of detectable changes in conduction velocity.

In-Phase Bilateral Exercise Effects on CMCT, MEPs amplitude and latency

Following visual analysis of the CMCT measurements, no significant improvement (i.e., reduction) was observed in any of the participants, possibly due to high variability and low data stability across all study phases. Given that participants did not engage in any exercise during baseline, stability of the data during the baseline phase was expected to be observed. Conversely, an exercise-induced effect on CMCT during the intervention phase was expected. During the follow-up phase, no significant improvement was observed as because participants did not engage in an exercise regime during this period

was expected. Although study's findings indicated an observable stable baseline for the right (Participants A, C, E) and left (Participants B, E) upper limbs, there was no improvement during the intervention on either the left or the right side.

Chapter 2 included a pilot single-case study (n = 1) and a single-case concurrent multiple baseline design study (n = 5), which include a small number of participants. The small sample size could be a reason for not detecting a CMCT effect in any of the participants.

Since the first single-case study was a pilot study, more comprehensive analysis can be performed for the single-case concurrent multiple baseline study. The participants (i.e., C and D for the left upper limb; A, B, C and E for the right upper limb) who showed an improvement (i.e., reduction of CMCT) between baseline and intervention phases, also showed unexpected trend directions (i.e., upward/downward) within study phases. The observed variability in trend directions, likely stems from inconsistent data across the study phases, may be attributed to factors such as manual coil positioning without neuronavigation system (Pellegrini et al., 2018) and the use of varying Transcranial Magnetic Stimulation (TMS) intensities (i.e., rMT was assessed on each session) per session (Herwig et al., 2001). Furthermore, the variability of TMS intensities used in each session could be another reason for such low data stability. For example, Pellegrini et al. (2018), discuss that different TMS intensities may activate different corticospinal pathways (Pellegrini et al., 2018), whereas Di Lazzaro and Rothwell (2014), indicated that high stimulus intensities result to later descending volleys (i.e., *I*- waves) (Di Lazzaro & Rothwell, 2014). Another contributor to low data stability could be the fact that corticospinal integrity in some pwMS can be affected during the very early disease stage (Pawlitzki et al., 2017), resulting in slower or blockage of the conduction time (i.e., prolonged CMCT) (Currà A et al., 2002).

Furthermore, no effect was found in motor evoked potentials (MEPs) amplitude and latency, possibly due to low data stability (i.e., < 80%) and unexpected trend directions (i.e., upward/downward). It is well established that MEPs amplitude is highly variable between-subjects and within-subjects in the same trial or on repeated trials (Goetz et al., 2014; Mills, 2004; Roy C et al., 2011). Another possible physiological explanation for not observing differences in the values of MEPs amplitudes and latencies is the high rMT. For example, Caramia et al. (1991), observed that individuals with a high rMT exhibited altered MEPs (Caramia et al., 1991). Similarly, in the current study, participants with a

high rMT demonstrated changes in MEPs sizes. Moreover, biological variables including the asynchronous firing of motor units, variations in cortical volume and differences in skull thickness may contribute to the observed low stability of data both within and across subjects (Wassermann, 2002).

Therefore, in the absence of an exercise-induced effect on CMCT, MEPs amplitude and latency may indicate that the intervention protocol utilized does not significantly affect the structural integrity of the descending neural pathways. It is hypothesized that the exercise protocol may have a stronger effect on the rMT than on the CMCT. This inference is based on the notion that in-phase bilateral exercises predominantly engage transcallosal mechanisms (McCombe Waller & Whithall, 2008; Ruddy et al., 2017) and interhemispheric interactions (McCombe Waller & Whithall, 2008).

In-Phase Bilateral Exercise Effects on Resting Motor Threshold

Since no significant changes were observed in CMCT, MEPs amplitude or latency, a key finding of pwRRMS is the bilateral decrease in rMT due to in-phase bilateral upper limb exercises. Considering that the rMT is a critical index of corticospinal excitability (Caramia et al., 2004; Hallett, 2007) and a lower threshold suggests higher cortical excitability (Veldema et al., 2021), these findings align with the initial hypothesis: in-phase bilateral exercises have a bilateral influence on the rMT.

Three out of four participants with RRMS, exhibited a bilateral decrease in rMT. However, one participant showed a decrease only in the left (non-dominant) upper limb. This bilateral decrease suggests an enhancement in rMT (Delvendahl et al., 2012; Malcolm et al., 2006), which it is attributed to the exercise regimen. Contrary to previous reports by Aramaki et al. (2006), and Neva et al. (2012), which found no change in rMT following antiphase bilateral exercises on the upper limbs in chronic stroke survivors (Aramaki et al., 2006; Neva et al., 2012), these data suggest that in-phase bilateral exercises can induce bilateral changes in pwRRMS.

Baseline measurements revealed a lower rMT in the right (dominant) upper limb compared to the left, corroborating findings from earlier studies (Aramaki et al., 2006; De Gennaro et al., 2004) in healthy individuals. Interestingly, a more pronounced decrease in the rMT was observed in the left (non-dominant) limb during our exercises, mirroring the effects reported by Waller et al. (2008), in chronic stroke survivors, where

the non-dominant limb experienced more significant improvements post-training (McCombe Waller & Whithall, 2008). This suggests enhanced interhemispheric facilitation, particularly in the hemisphere corresponding to the non-dominant limb.

Previous research in chronic stroke survivors (Cauraugh & Summers, 2005; Stinear & Byblow, 2002) has indicated the role of transcallosal pathways in modulating bilateral cortical excitability. Activation of these pathways typically involves a concurrent decrease in interhemispheric inhibition and an increase in intracortical facilitation (Neva et al., 2012; Smith & Richard Staines, 2010; Stinear & Byblow, 2002). Luft et al. (2004), demonstrated that in-phase bilateral training activated cortical regions such as the ipsilesional precentral gyrus and contralesional superior frontal gyrus in stroke survivors, as shown by functional MRI (Luft et al., 2004). Additionally, Whitall et al. (2011), highlighted the effectiveness of in-phase bilateral exercises for enhancing corticospinal plasticity in chronic stroke survivors (Whitall et al., 2011). My research extends these findings in pwRRMS, proposing that in-phase bilateral training can also enhance cortical plasticity and clinical outcomes in this clinical population.

While aerobic training is known to improve corticospinal plasticity (Kuo et al., 2023; Mark et al., 2023; Stellmann et al., 2020), it is essential to differentiate the effects of exercise intensity from those of the movement mechanism. To this end, a constant heart rate (HR) was maintained below the aerobic threshold (70% of the maximum HR (Skinner & McLellan, 1980)) to ensure that my observations stemmed from the specific in-phase bilateral mechanism and not general exercise effects.

However, high variability was observed in the data for Participant D and the right limb of Participant A. This variability could relate to individual disease progression, as these participants are nearing a decade since the onset of RRMS—a point at which many transitions to a progressive stage (Correale et al., 2017; Correale & Marrodan, 2019). Such progression may account for the observed neural excitability changes (Louapre & Lubetzki, 2015; Lubetzki & Stankoff, 2014; Trapp & Nave, 2008). Lastly, the specific study design did not anticipate exercise-induced effects during the follow-up phase, as no exercise was prescribed. A descriptive analysis of this phase revealed a bilateral trend towards baseline levels, starting from the first month post-intervention. An increase in the rMT during follow-up—when exercise was absent—suggests a reversible effect on

corticospinal plasticity (Delvendahl et al., 2012) and underscores the potential benefit of the specific exercise protocol.

In-Phase Bilateral Exercise Effects on Clinical Measures

Beyond these neurophysiological insights, the intervention resulted in observable improvements in clinical measures in both pwRRMS and pwPMS. These enhancements spanned multiple domains, including motor function and cognitive processing, highlighting the potential therapeutic benefits of the exercise regimen for both clincial cohorts.

During the intervention phase performed in pwRRMS, improvements across all clinical metrics were observed, while the follow-up phase was characterized by a regression to pre-intervention conditions, suggesting the absence of sustained benefits from the exercise protocol. Nonetheless, the temporary enhancements during the intervention highlight the potential efficacy of the exercise regimen on clinical outcomes for pwMS.

Continuous engagement in exercise is well-documented to ameliorate a range of clinical symptoms in Multiple Sclerosis (MS) patients (DeLuca et al., 2020; Kalb et al., 2020; Reina-Gutiérrez et al., 2022). The exercise protocol in the first clinical trial, which emphasized bilateral upper limb movements, led to measurable enhancements in manual dexterity and limb strength, as evidenced by improved scores in Action Research Arm Test and the muscle strength test, respectively. This enhancement is indicative of improved upper limb function, which is beneficial across various MS subtypes (Lamers & Feys, 2014). Additionally, all five participants exhibited improvements in balance and dynamic gait, as measured by the Mini-Balance Evaluation Systems Test and the Six Spot Step Test. While the exercise regimen primarily involved the upper limbs, the observed benefits in balance and gait likely stemmed from the circuit training structure of the physical activity (Chisari et al., 2014; Novotna K et al., 2019), which inadvertently involved gait and balance practice through transitions between exercises.

Although information processing speed is the predominant clinical symptom in PMS, which was the primary research question in the second study of this thesis, an improvement in information processing speed was also observed in pwRRMS. These results align with findings by Sandroff et al. (2016), who demonstrated cognitive improvements in MS patients following diverse exercise protocols (Sandroff, Motl, et al.,

2016). Notably, the present study recorded simultaneous enhancements in motor skills—including balance, gait, and hand dexterity—and cognitive speed, supporting literature that underscores the interdependence of cognitive functions and bimanual coordination (Rudisch et al., 2020; Vasylenko et al., 2018b, 2018a; Yozbatiran et al., 2008) and their collective impact on physical disability risk in MS (Hechenberger et al., 2022).

Visual analysis of the data from Modified Fatigue Impact Scale and Medical Outcomes Study Questionnaire Short Form 36, revealed discernible changes in individual QoL and fatigue levels, encompassing physical, cognitive, and psychosocial domains, during both intervention and follow-up phases relative to baseline. These findings are consistent with prior research indicating that improved clinical status in MS is often correlated with heightened fatigue perception (Ayache & Chalah, 2017; Induruwa et al., 2012; Razazian et al., 2020), substantiating the notion that clinical improvements in these participants could be associated with the observed alterations in fatigue levels.

The first clinical trial study represents an initial effort to investigate the impact of in-phase bilateral upper limb exercises on corticospinal plasticity in pwRRMS. To the best of our knowledge, it is the first of its kind to explore this area. The current study has provided compelling evidence that in-phase bilateral upper limb exercises can modulate the rMT, a measure known for its simplicity and reliability in assessing neural plasticity (Delvendahl et al., 2012; Julkunen P et al., 2011; Malcolm et al., 2006; Schutter D & Jack van Honk, 2006). These findings lay the groundwork for further exploration of the rMT as a prognostic tool for corticospinal plasticity in MS. Additionally, the observed improvements in various clinical measures endorse the potential of in-phase bilateral upper limb exercises as a viable rehabilitation approach for enhancing the clinical outcomes of those with RRMS.

While the benefits of in-phase bilateral exercises for pwRRMS are promising, progressive forms of MS, present more complex challenges. These forms of MS are characterized by a gradual and sustained worsening of neurological function, including motor and cognitive impairments making rehabilitation efforts, including motor and cognitive interventions, more challenging.

4.1.2 The Effects of In-Phase Bilateral Exercises in Progressive Multiple Sclerosis

Although rehabilitation interventions in RRMS focus on regaining mobility by enhancing corticospinal plasticity, the approach differs significantly in PMS, where physiotherapy prioritizes task-specific training and cognitive strategies to maintain independence. In RRMS, rehabilitation aims to promote neuroplasticity and functional recovery, helping individuals regain lost motor function following relapses. In contrast, PMS physiotherapy approaches focus on both motor and cognitive training to slow functional decline and optimize remaining abilities. While both forms of MS benefit from individualized physiotherapeutic plans, the key distinction lies in stimulating neural adaptation for recovery in RRMS versus preserving function through motor-cognitive training in PMS.

The second clinical trial study of this thesis examined the effects of a 12-week in-phase bilateral upper limb exercise program primarily on information processing speed and, secondarily, on manual dexterity, dynamic balance, gait, fatigue, and QoL in pwPMS. The specific exercise program significantly improved information processing speed and the other secondary measures compared to conventional exercises, supporting the primary hypothesis. However, the secondary hypothesis of this clinical trial—that improvements in information processing speed would enhance manual dexterity—was not supported, as the observed correlation was not statistically significant.

In-Phase Bilateral Exercise Effects on Information Processing Speed

Statistical analysis of the Symbol Digit Modalities Test revealed significant improvement (i.e., increased scores) in both the experimental and active control groups, indicating enhanced information processing speed in pwPMS (Benedict et al., 2020; Costa et al., 2017). Since participants in both groups were not engaged in any other exercise program prior to the intervention phase, the baseline data remained stable. While exercise-induced improvements in the Symbol Digit Modalities Test were observed during the intervention phase in both groups, the experimental group demonstrated a statistically significant greater improvement compared to the active control group. This finding was also observed in pwRRMS in the first clinical trial study, as the same exercise protocol was used during the intervention phase.

Information processing speed is recognized as an independent prognostic factor for physical impairment (Hechenberger et al., 2022) and the most common cognitive dysfunction (Benedict et al., 2020; Bergendal et al., 2007; DeLuca et al., 2020), in pwPMS. Our findings align with previous studies demonstrating the positive impact of exercise on information processing speed in this clinical population (Coote et al., 2017; Morrison & Mayer, 2017; Sandroff, Balto, et al., 2016; Sandroff et al., 2018; Sosnoff et al., 2017). Additionally, the results from this study corroborate findings from prior the first clinical trial study in RRMS (see Chapter 2.2) (Sokratous et al., 2023), which reported improvements in cognitive processing the same type of exercises.

Research studies in the field of cognitive-motor connections are becoming increasingly frequent, as a strong relationship has been established between cognitive function and upper limb performance in both healthy individuals and those with MS (Einarsson et al., 2006; Kierkegaard et al., 2012; Lamers & Feys, 2014; Raats et al., 2018; Yozbatiran et al., 2006). The anterior cingulate cortex plays a critical role in cognition, including decision-making, attention, emotion regulation, and cognitive processing (Fellows & Farah, 2005; Friedman & Robbins, 2022). Notably, functional connectivity between cognitive and motor functions has been documented, mediated by dense projections from the anterior cingulate cortex to the motor cortex and spinal cord (Asemi et al., 2015; Bush et al., 2000; Paus, 2001). Specifically, the dorsal division of the anterior cingulate cortex, known as the dorsal cognitive division (Figure 1.18), is activated during motor tasks and contributes to cognitive processing regulation (Asemi et al., 2015; Bush et al., 2000; Van Veen & Carter, 2002).

Asemi et al. (2015), demonstrated the functional relationship between the dorsal division of the anterior cingulate cortex and the motor system, highlighting its role in controlling the SMA in healthy individuals. Moreover, previous research has shown that bimanual hand coordination is linked to symmetrical facilitation of neural activity through increased intrahemispheric connectivity and greater transcallosal coupling between the SMA and the M1 (Asemi et al., 2015; Grefkes et al., 2008; Rudisch et al., 2020; Swinnen & Wenderoth, 2004). In particular, Grefkes et al. (2008), highlighted the efficacy of in-phase bilateral upper-limb movements in enhancing interhemispheric connectivity between the SMA and M1 in both brain hemispheres (Grefkes et al., 2008) (Figure 1.19).

Therefore, the current exercises protocols demonstrate that can effectively improve information processing speed in pwPMS, as wells as in pwRRMS. These findings align with the theory that the anterior cingulate cortex plays a crucial role in coordinating motor actions and processing motor-related information through its functional connectivity with motor skills. This mechanism further supports the integration of bilateral upper-limb exercises into rehabilitation programs for pwPMS to address a range of needs, including cognitive and motor impairments.

In-Phase Bilateral Exercise Effects on Manual Dexterity

Alongside with improvement on information processing speed, the proposed exercise protocol showed a statistically significant difference in manual dexterity measures between the experimental and active control groups, in pwPMS. Notably, significant differences were observed in the baseline phases between subtests within both study groups. Participants in both groups performed better in the Unimanual Dominant subtest, as measured by the number of pegs, compared to the other subtests. This difference can be attributed to the involvement of the non-dominant hand in the other three subtests (Unimanual Non-Dominant, Bimanual, and Assembly), which increased task difficulty and required greater effort.

Although the experimental group began with higher baseline scores across all Purdue Pegboard subtests compared to the active control group—likely due to the stronger initial performance of participants in the experimental group despite partial random allocation—a post hoc statistical analysis with Bonferroni correction revealed that these baseline differences were not statistically significant. This indicates that both groups had comparable starting points, reducing the potential impact of baseline differences on the study outcomes.

Furthermore, since participants in the experimental group demonstrated statistically significant improvements compared to the control group across all Purdue Pegboard subtests, this indicates that the proposed in-phase bilateral exercise program has a greater impact on enhancing manual dexterity in these individuals.

Results from the Purdue Pegboard test indicated no statistically significant improvement in any subtests for the active control group. However, participants in the experimental group achieved higher scores in the Unimanual Dominant, Bimanual, and Assembly

subtests. Although the experimental group also showed improvement in the Unimanual Non-Dominant subtest, this improvement was not statistically significant. These findings align with previous studies that reported bilateral practice positively affects both the dominant and non-dominant sides of the body due to positive learning transfer between limbs (Aune et al., 2017; Focke et al., 2016). Bilateral practice helps reduce lateral asymmetries, which otherwise cause performance discrepancies between the two sides of the body (Teixeira, 2003).

Additionally, significant improvements were observed in both the Bimanual and Assembly subtests of the Purdue Pegboard test among participants in the experimental group, with the greatest improvement noted in the Assembly subtest. This is likely due to the specific nature of the exercise program—in-phase bilateral—. These effects may be attributed to the functional role of the corpus callosum (Gooijers & Swinnen, 2014) (Gooijers & Swinnen, 2014) and enhances unilateral upper limb performance (Smith & Richard Staines, 2010),

The improvements in the Bimanual and Assembly subtests of the Purdue Pegboard Test align with research highlighting the role of interhemispheric communication and motor coordination (Lee & Kang, 2023). Studies emphasize that the specific test, widely used for assessing bimanual dexterity, reliably detects motor deficits and changes in coordination through tasks requiring simultaneous use of both hands. Bilateral training, including exercises that emphasize interhemispheric interactions, has been shown to improve motor control and reduce asymmetries, supporting its therapeutic potential for neurorehabilitation. These insights are consistent with findings in neurorehabilitation, where bilateral and bimanual exercises have demonstrated benefits in enhancing motor learning and adaptability, particularly in individuals with neurological conditions (Gerloff & Andres, 2002; McCombe Waller & Whithall, 2008; Morita et al., 2023).

The studies of Seitz et al. (2004) and Smith and Staines (2010), reported that visual cue in-phase bimanual training increases cortical activity, and it also improves in-phase bimanual dexterity performance in both clinical and healthy populations (Seitz et al., 2004; Smith & Richard Staines, 2010). Considering that lesions within the corpus callossum may be occur in pwMS, leading to difficulties in bimanual coordination (Bonzano et al., 2008; Larson et al., 2002) and since results from the current study align with those of Seitz et al. (2004) and Smith et al. (2010) (Seitz et al., 2004; Smith &

Richard Staines, 2010), the present study confirms that in-phase bilateral exercises of the upper limbs could enhance bimanual dexterity in PMS.

The lack of statistically significant improvement in the Unimanual Non-Dominant subtest for the experimental group may be attributed to the nature of the exercise program—in-phase bilateral—which may have inadvertently encouraged greater effort from the dominant hand than the non-dominant hand. According to the literature, significant changes and skill acquisition depend on task-specific exercises that enhance neuroplasticity in the targeted limb, particularly the non-dominant one. Additionally, the duration and frequency of the exercise program (i.e., three months with three sessions per week) might have been insufficient to detect significant changes in the non-dominant side. Previous studies suggest that programs lasting up to 16 weeks with higher session frequencies (three to five sessions per week) are often necessary to achieve measurable improvements in the non-dominant limb due to its lower baseline performance and greater need for neural adaptation (Dayan & Cohen, 2011; El-Sayes et al., 2019; Pickersgill et al., 2022). Nevertheless, the observed bilateral improvement indicates that this exercise program could still be valuable for populations that often neglect or exclude the non-dominant or affected upper limb in their training, such as those with motor impairments or disabilities.

In-Phase Bilateral Exercise and The Relationship Between Information Processing Speed and Manual Dexterity

To investigate the secondary hypothesis of the second clinical trial—that the improvement in information processing speed resulting from the exercise protocol (in-phase bilateral exercises) affects manual dexterity in the experimental group—a Pearson's correlation analysis was conducted between the Symbol Digit Modalities Test and the Purdue Pegboard sub-tests. Although a moderate correlation was observed between these two outcome measures, only the correlation between Symbol Digit Modalities Test and the assembly subtest was found to be significant (see Tables 3.4 and 3.5).

Throughout the correlation analysis a strong relationship was observed between information processing speed and manual dexterity in the participants of the experimental group, which was not observed in the active control group. These findings align with previous studies that have demonstrated a similar relationship between these two

variables in pwPMS (Kierkegaard et al., 2012; Rudisch et al., 2020; Yozbatiran et al., 2006), those with in mild cognitive impairment individuals and healthy older adults (Rudisch et al., 2020; Vasylenko et al., 2018b).

Especially the assembly subtest which is the most complex task of the Purdue pegboard test, indicated the greater improvement in participants of the experimental group, compared to the other subtests. These findings confirm our secondary hypothesis, that in-phase bilateral upper limbs exercises could improve manual dexterity alongside with improvement of cognitive processing in the participants of the experimental group. The assembly subtest requires higher-order cognitive control (Rodríguez-Aranda et al., 2016; Strenge, 2002), as participants need to recognize the sequential order to place the required object (i.e., pegs, collars, washers), with a specific hand (i.e., dominant and non-dominant hands), within a limited time. On the contrary, these behavioral relationships were not evident for the participants of the active control group, in which they performed conventional exercises.

In-Phase Bilateral Exercise Effects on Clinical Measures

In addition to improvements in information processing speed and manual dexterity, a statistically significant improvement was observed in the participants of the experimental group on all secondary outcome measures, compared to the active control group. Although the specific exercise program primarily targeted the upper limbs, the observed benefits in gait and dynamic balance were likely due to the circuit training structure of the physical activity (Chisari et al., 2014; Novotna K et al., 2019), which encompassed both gait and balance practice through transitions between exercises.

As it was also reported in pwRRMS, the specific type of exercises exhibited improvements in fatigue level and QoL in pwPMS. Following the statistical analysis of the Medical Outcomes Study Questionnaire Short Form 36 Health Survey and the Modified Fatigue Impact Scale, a significant improvement was indicated in the QoL and fatigue level only in the experimental group. These findings are consistent with prior research indicating that improved cognitive functions and manual dexterity in MS, is often associated with increase fatigue perception (Andreasen et al., 2010, 2019; Diamond et al., 2008; Kamm et al., 2015) and improvement of individuals' QoL (Baumstarck-Barrau et al., 2011; Cutajar et al., 2000; Glanz et al., 2010).

Over the past few decades, exercise has emerged as a key component in managing cognitive and motor dysfunctions in both RRMS and PMS. Considering future directions from De Luca et al. (2020), who recommended that future research should be focused on the effects of a holistic training paradigms and targeting specific cognitive domains (DeLuca et al., 2020). According to that, both studies' methodologies focused on the impact of a specific type of exercise in specific outcome measures. Additionally, the second clinical trial study has provided compelling evidence that in-phase bilateral upper limb exercises can improve bimanual hand coordination, a motor skill known for its complexity in terms of high-order cognitive control. Furthermore, in-phase bilateral upper limb exercises highlighted the potential for targeted exercise interventions to yield clinically meaningful improvements and indicated a correlation between information processing speed and manual dexterity. However, further investigation of the relationship between cognitive processing and manual dexterity should be evaluated in larger studies.

Accordingly, since cognitive and motor dysfunctions are the most common clinical symptoms in pwPMS and given that in-phase bilateral exercises needs less attentional load and less control than unilateral exercises (Koeneke et al., 2004; Swinnen, 2002; Temprado et al., 1999), the observed improvements in the information processing speed and on manual dexterity measures, recommend the specific type of exercise as a reliable rehabilitation approach in pwMS. The current exercise protocols were based on a holistic approach, incorporating sports activities and functional exercises within a group circuit training format. Acknowledging the limitations, this research provides preliminary insights into the impact of this type of exercise on both motor and cognitive functions in pwPMS and pwRRMS.

4.2 Interpretation of Findings

The findings of this thesis highlight the impact of in-phase bilateral upper limb exercises on neurophysiological, cognitive and clinical outcomes in pwMS.

Since prolongation of CMCT is the most common neurophysiological characteristic in pwRRMS, the in-phase bilateral exercise protocol did not significantly alter CMCT, indicating no changes in the structural integrity or conduction velocity of the corticospinal tract. However, a bilateral reduction in rMT was observed, which reflects enhanced cortical excitability—a key marker of neuroplasticity. This bilateral rMT decrease,

particularly pronounced in the non-dominant hemisphere, suggests increased corticospinal excitability.

These results align with evidence that rMT changes are associated with neuroplastic adaptations and improvement of functional outcomes in people with neurological disorders (De Las Heras et al., 2024; Veldema et al., 2021). The findings further support the hypothesis that in-phase bilateral exercises engage interhemispheric pathways via transcallosal mechanisms (Welniarz et al., 2015) enhancing communication and coordination between the hemispheres.

The observed greater reduction in rMT compared to CMCT highlights the predominance of interhemispheric facilitation over changes in conduction speed along corticospinal tracts in pwRRMS. This suggests that in-phase bilateral exercises might preferentially modulate cortical excitability and connectivity rather than merely altering the integrity or speed of peripheral conduction. Furthermore, the more pronounced reduction in rMT in the non-dominant limb suggests enhanced facilitation originating from the dominant hemisphere (Takeuchi et al., 2012). This asymmetric facilitation aligns with patterns observed in stroke rehabilitation, where the dominant hemisphere often compensates for deficits in the non-dominant side by exerting increased influence over interhemispheric pathways (Hayward et al., 2017; Rosso & Lamy, 2018; Takeuchi et al., 2012). This phenomenon could reflect a broader principle of motor control and recovery, wherein the dominant hemisphere acts as a critical driver of neuroplastic changes in bilateral motor networks.

Despite the absence of measurable changes in CMCT, participants experienced improvements in both motor and cognitive functions. This suggests that in-phase bilateral exercises produce clinically meaningful effects that extend beyond direct corticospinal modulation. These findings are consistent with the broader therapeutic benefits of bilateral exercises documented in the neurorehabilitation literature (Chen et al., 2019).

During in-phase bilateral hand movements, where both M1 areas are activated simultaneously, a positive coupling between M1 and the SMA occurs, enhancing connectivity within the motor network. This simultaneous activation promotes positive neural modulation and strengthens interhemispheric facilitation, which ultimately improves motor function. This is significant, as changes in corticospinal excitability across both hemispheres can be observed after an acute relapse in pwRRMS.

Together, these results underscore the importance of designing therapeutic interventions that leverage interhemispheric mechanisms and dominant-hemisphere facilitation to optimize recovery and functional outcomes. Future studies should focus on elucidating the precise neurophysiological processes underlying these effects, including the role of specific transcallosal circuits and the potential for targeting these pathways through adjunctive neuromodulation techniques.

In pwPMS, the in-phase bilateral upper limb exercise program led to statistically significant improvements in information processing speed, manual dexterity, and clinical outcomes compared to the active control group. These findings highlighted the significant translational potential of the in-phase bilateral upper limb exercise in enhancing information processing speed—a critical cognitive domain affected in pwPMS. As information processing speed is a known prognostic factor for physical impairment and the most common cognitive dysfunction in this population, these findings emphasize the clinical value of incorporating targeted exercise protocols into rehabilitation plans. Improved information processing speed may translate to better daily functioning, improved task efficiency and overall QoL for pwPMS.

The observed correlation between cognitive improvement and motor performance aligns with prior evidence that the anterior cingulate cortex plays a pivotal role in cognitive regulation and motor coordination. The functional connectivity between the anterior cingulate cortex, supplementary motor area and primary motor cortex, facilitated by bilateral training, suggests that in-phase bilateral exercises can enhance both domains simultaneously. This supports a holistic approach to MS rehabilitation, addressing both cognitive and motor impairments through shared neural pathways.

Improvements in the Bimanual and Assembly subtests (Purdue Pegboard Test) for the experimental group in pwPMS, highlight the role of in-phase bilateral exercises in enhancing coordination through interhemispheric communication facilitated by the corpus callosum. This aligns with prior research highlighting the benefits of bilateral training in promoting positive learning transfer between limbs and minimizing lateral asymmetries, thereby enhancing motor function and coordination across both hemispheres (Gnanaprakasam et al., 2023; Kidgell et al., 2017).

Additionally, the correlation between information processing speed (as measured by the Symbol Digit Modalities Test) and the Assembly subtest suggests that in-phase bilateral

exercises not only enhance motor skills but also improve cognitive processing, particularly for tasks requiring higher-order control. The Assembly subtest, which involves sequential planning and dual-hand coordination, proved to be the most sensitive assessment tool for detecting the relationship between cognitive and motor improvements.

Finally, it seems that training in a circuit format which includes translation movements, despite focusing on upper limb exercises, both pwRRMS and pwMS in the experimental group showed improvements in all clinical outcomes (gait, balance, fatigue and QoL) This finding underscores the holistic benefits of in-phase bilateral training.

The absence of adverse effects, coupled with the demonstrated superiority of in-phase bilateral exercises over conventional programs in improving corticospinal plasticity, cognitive processing, and broader clinical outcomes underscores the feasibility and potential utility of in-phase bilateral exercises as a therapeutic intervention for pwMS. These findings highlight the holistic benefits of in-phase bilateral training, offering a strong basis for its integration into neurorehabilitation programs to address the complex interplay between cognitive and motor dysfunctions in MS.

4.3 Study Limitations

The current thesis presents several methodological considerations that should be overcome in future research.

1. The limited sample size for both clinical trials, while sufficient for pilot studies aimed at generating initial data to guide future research, may affect the generalizability of our results. To mitigate this, in the first clinical trial study (see Chapter 2) a concurrent multiple baseline design (Kratochwill et al., 2010) was employed, which allows for systematic and individualized data collection at multiple time points, enhancing the robustness of the findings despite the sample size. In the second clinical trial study (see Chapter 3), twenty participants were allocated (1:1) to either the experimental or active control group, enabling a reliable interpretation of the results through both within-group and between-group statistical analyses. Additionally, based on the observed mean difference between the two groups, the sample size reached 100% statistical power to detect these effects for the primary outcome measure (i.e., information processing speed). Therefore, these findings can serve as a basis for larger studies.

2. TMS assessments were performed using traditional methods without neuronavigation system. Although use of neuronavigation system can enhance the precision of stimulating consistent motor cortex areas (Bardel et al., 2024; Jung et al., 2010), the reproducibility and variability of TMS measures are not necessarily compromised by the absence of such technology (Bastani & Jaberzadeh, 2012). To ensure the reliability of the TMS data, there was a systematic data collection and data analysis from 30 suprathreshold stimuli for each participant (Sivaramakrishnan & Madhavan, 2020), implementing stringent criteria to calculate mean values for each data point, thereby maintaining the integrity of the TMS assessments.

3. The use of Abductor Pollicis Brevis for the electromyogram data collection to assess corticospinal excitability of the upper limbs in the first clinical trial study (see Chapter 2) of this thesis. This choice, while standard, does not encompass the activity of other muscles such as the biceps brachii, or the wrist flexors and extensors, which may be more directly engaged with our exercises and could therefore provide a more nuanced view of corticospinal excitability changes due to their distinct biomechanical contributions to upper limb movements.

4. The absence of a follow-up phase in the second clinical trial study (see Chapter 3). Follow-up assessments are essential for ensuring the integrity and reliability of findings and can provide valuable insights into potential long-term effects on participants. Since the primary objective of this study was to determine whether this specific type of exercise influences cognitive processing, a follow-up phase was not included. However, future studies that incorporate follow-up assessments could offer insights into the sustained benefits of this exercise protocol, particularly its long-term effects. This could suggest that pwMS may continue to experience benefits even during periods when they are not actively exercising.

5. The duration of the intervention phases and the frequency of the exercise sessions for both study groups, in the second clinical trial. On the one hand the 12-weeks intervention phases could be a short period to designate notable cognitive effects in pwPMS. This may be a possible reason why we did not observe a significant improvement in participants of the active control group. On the other hand, the difference in training frequency between the two groups (i.e., experimental group: 3x/week, control group: 1x/week) could result in a significant effect only in the experimental group. As is well known, higher exercise

dosage and greater frequency lead to more significant improvements in pwMS (Stroud & Minahan, 2009) and in healthy individuals (Kemmler & Von Stengel, 2013; Nakamura et al., 2007). Nonetheless, the methodology used in this study addresses a crucial research gap, as noted by De Luca et al. (2020), who pointed out the lack of studies examining the optimal frequency and duration of interventions for pwMS (DeLuca et al., 2020). It is acknowledged that since the exercise dosage differs between the two study groups, one might argue that the study primarily compares exercise quantity rather than the mechanism behind the specific types of exercises. However, as evidenced by the within-experimental group analysis, a statistically significant improvement in all outcome measures was observed from baseline to intervention, compared to the within-active control group analysis. This limitation underscores the need for future studies that can address this gap.

6. Since the participants in the experimental group, in the second clinical trial (see Chapter 3), were trained specifically on upper-limb exercises, while those in the active control group followed a more general exercise program, the experimental group's manual dexterity performance may have shown greater improvement. This difference could represent a limitation of our study, particularly with regard to our secondary hypothesis, where we aimed to establish a correlation between cognitive processing and manual dexterity.

7. The review study by De Luca et al. (2020), defined the influence of a baseline cognitive status to the intervention response (DeLuca et al., 2020). This could be a limitation for the second clinical trial (see Chapter 3) of the present thesis, given that there was a difference in the baseline status between experimental and active control group. Even though there was a partial randomized allocation of the participants, the participants of the experimental group had better scores in most of the outcome variables. To overcome this limitation, except for the between group comparison, a within group comparison was performed to investigate the improvement within each group. Accordingly, the results showed greater improvement to all outcome measures on the experimental group, compared to the active control group, confirming the hypotheses.

8. In the second clinical trial (see Chapter 3) of the present thesis, there was no monitoring of the individuals' HR during the exercise sessions in both study groups, therefore this could possibly lead to a misinterpretation of the results. Given that aerobic exercise

enhances clinical condition of pwMS and since there was no HR monitor, there was a possibility of some participants to exercise within the aerobic level (i.e., 70% of individual maximum HR (Skinner & McLellan, 1980). However, to mitigate this, several simple exercises without big resistance and by monitoring individuals' fatigue level were involved in that specific exercise program. Prior to the intervention phase, all participants were instructed to report to their trainers, if any of them exceeded individual moderate fatigue level (i.e., RPE = 5).

4.4 Future Directions

The current thesis demonstrated the significant impact of in-phase bilateral upper limb exercises on neurophysiological, cognitive, and clinical outcomes in pwMS, while considering several methodological limitations. Although the results offer preliminary insights into the influence of exercise on corticospinal excitability, they contrast with those of Snow et al. (2019), who questioned the clinical relevance of motor threshold in MS due to a lack of reported correlations with clinical outcomes (Snow et al., 2019).

Furthermore, while the sample size of the second clinical trial study (see Chapter 3) reached 100% statistical power for detecting the effects of the primary outcome measure (i.e., information processing speed), these findings are still considered preliminary and can serve as a basis for larger studies. To reconcile these differing perspectives, future research should replicate the exercise protocol with a larger sample of pwRRMS to establish rMT as a crucial marker of corticospinal plasticity in this cohort, while also expanding the sample of pwPMS to confirm and generalize improvements in cognitive processing.

Additionally, given rMT's potential role in predicting rehabilitation outcomes (Rosso & Lamy, 2018; Veldema et al., 2021), further analysis comparing hemispheric data and their clinical correlations is warranted. This approach could provide valuable insights into the relationship between neural plasticity and patient recovery. Discrepancies in the literature regarding increased rMT in MS and its association with hemispheric dominance (Hayward et al., 2017; Simpson & Macdonell, 2015; Zipser et al., 2018) also require further investigation. Future studies should aim to clarify the connection between rMT and active motor threshold, as well as the relationship with hand dominance and clinical metrics.

To provide a more comprehensive understanding of rehabilitation progress, future research could expand the methodological toolkit to include various quantitative neuromechanical assessments, such as gait analysis, isokinetic dynamometry, and monitoring of HR and oxygen consumption. These measures would complement rMT and clinical outcomes to offer a fuller picture of neuromechanical function.

Moreover, future studies should explore the effects of in-phase bilateral exercises on other cognitive domains, such as working memory and attention. Investigating the correlation between information processing speed and other motor skills, alongside diverse outcome measures, would allow for a more holistic evaluation of rehabilitation progress in pwMS. Lastly, studies using functional magnetic resonance imaging should explore the hypothesis that in-phase bilateral exercises enhance communication and coordination between the upper limbs by activating both brain hemispheres.

4.5 Conclusion

This thesis investigates the impact of in-phase bilateral upper limb exercises on neurophysiological, cognitive, and clinical outcomes in pwMS.

Since prolongation of CMCT is the most common neurophysiological characteristic in pwMS, in-phase bilateral exercises did not significantly alter CMCT in pwRRMS, suggesting no changes in corticospinal tract structural integrity or conduction velocity. However, bilateral reductions in rMT were observed, reflecting enhanced cortical excitability, a marker of neuroplasticity. The pronounced rMT reduction in the non-dominant hemisphere suggests enhanced corticospinal excitability and interhemispheric facilitation, mediated by transcallosal mechanisms. The findings indicate that in-phase bilateral exercises primarily affect interhemispheric pathways rather than corticospinal tract conduction speed, consistent with evidence from stroke rehabilitation.

Although the CMCT remained unchanged in pwRRMS, improvements in motor and cognitive function were evident, supporting the clinical relevance of in-phase bilateral exercises in neurorehabilitation. These findings support the theory that during in-phase bilateral movements, both M1 areas are activated simultaneously, creating a positive coupling between M1 and the SMA groups (Gerloff & Andres, 2002). This simultaneous activation promotes positive neural modulation, leading to improved motor function.

Given that cognitive and motor dysfunctions are the primary clinical symptoms in pwPMS, the in-phase bilateral exercise protocol led to significant improvements in information processing speed, manual dexterity, and clinical outcomes, outperforming conventional exercise programs. Improvements in Symbol Digit Modalities Test scores underscore the efficacy of in-phase bilateral exercises in enhancing information processing speed, a critical prognostic factor for physical impairment and the most common cognitive dysfunction in pwPMS. Enhanced performance in Purdue Pegboard Test subtests (Bimanual and Assembly) highlights the role of in-phase bilateral exercises in improving interhemispheric communication via the corpus callosum, reducing lateral asymmetries, and facilitating positive learning transfer between limbs. A significant correlation between information processing speed and Assembly subtest performance demonstrates the interplay between cognitive and motor improvements, particularly in tasks requiring higher-order cognitive control.

Despite focusing on upper limb exercises, participants showed improved gait, dynamic balance, reduced fatigue and enhanced QoL. These benefits suggest that the holistic structure of in-phase bilateral training, including transitional movements, contributes to broader clinical improvements.

The absence of adverse effects and the demonstrated superiority of in-phase bilateral exercises over conventional programs affirm their feasibility and utility as a therapeutic intervention for pwMS. The findings provide strong evidence for the integration of in-phase bilateral exercises into neurorehabilitation to address the complex interplay of cognitive and motor dysfunction in MS.

The current thesis highlights several methodological considerations that should be addressed in future research. First, the limited sample sizes in both studies, while appropriate for pilot research aimed at generating preliminary data, may limit the generalizability of the findings. Second, TMS assessments in the first clinical trial (see Chapter 2) were conducted using traditional methods without neuronavigation system, which could enhance the precision of cortical stimulation. Third, the absence of a follow-up phase in the second clinical trial (see Chapter 3) limits the ability to assess the reliability of the findings and their potential long-term effects. Fourth, the duration of the intervention phases and the frequency of exercise sessions varied between study groups in the second clinical trial (see Chapter 3), which may have influenced the outcomes.

Finally, there was no monitoring of participants' HR during exercise sessions in either group, which could have impacted the consistency of exercise intensity across participants. These considerations underline the need for refined methodologies in future research to enhance the reliability and applicability of findings.

Future research should explore long-term effects and the underlying mechanisms driving these improvements to optimize rehabilitation strategies further. This work establishes a solid foundation for using in-phase bilateral upper limb exercises as a holistic and effective approach in MS care.

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APPENDIX I

Functional Systems

1. Pyramidal Functions

Score	Description
0	Normal.
1	Abnormal signs without disability.
2	Minimal disability.
3	Mild or moderate paraparesis or hemiparesis; severe monoparesis.
4	Marked paraparesis or hemiparesis; moderate quadriparesis; or monoplegia.
5	Paraplegia, hemiplegia, or marked quadriparesis.
6	Quadriplegia.
V	Unknown.

2. Cerebellar Functions

Score	Description
0	Normal.
1	Abnormal signs without disability.
2	Mild ataxia.
3	Moderate truncal or limb ataxia.
4	Severe ataxia, all limbs.
5	Unable to perform coordinated movements due to ataxia.
V	Unknown.
X	Is used throughout after each number when weakness (grade **3** or more on pyramidal) interferes with testing.

3. Brain Stem Functions

Score	Description
0	Normal.
1	Signs only.
2	Moderate nystagmus or other mild disability.
3	Severe nystagmus, marked extraoccular weakness, or moderate disability of other cranial nerves.
4	Marked dysarthria or other marked disability.
5	Inability to swallow or speak.
V	Unknown.

4. Sensory Functions

Score	Description
0	Normal.
1	Vibration or figure-writing decrease only, in one or two limbs.
2	Mild decrease in touch or pain or position sense, and/or moderate decrease in vibration in one or two limbs; or vibratory (c/s figure writing) decrease alone in three or four limbs.
3	Moderate decrease in touch or pain or position sense, and/or essentially lost vibration in one or two limbs; or mild decrease in touch or pain and/or moderate decrease in all proprioceptive tests in three or four limbs.
4	Marked decrease in touch or pain or loss of proprioception, alone or combined, in one or two limbs; or moderate decrease in touch or pain and/or severe proprioceptive decrease in more than two limbs.

5	Loss (essentially) of sensation in one or two limbs; or moderate decrease in touch or pain and/or loss of proprioception for most of the body below the head.
6	Sensation essentially lost below the head.
V	Unknown.

5. Bowel and Bladder Functions

Score	Description
0	Normal.
1	Mild urinary hesitancy, urgency, or retention.
2	Moderate hesitancy, urgency, retention of bowel or bladder, or rare urinary incontinence.
3	Frequent urinary incontinence.
4	In need of almost constant catheterization.
5	Loss of bladder function.
6	Loss of bowel and bladder function.
V	Unknown.

6. Visual (or Optic) Functions

Score	Description
0	Normal.
1	Scotoma with visual acuity (corrected) better than 20/30.
2	Worse eye with scotoma with maximal visual acuity (corrected) of 20/30 to 20/59.
3	Worse eye with large scotoma, or moderate decrease in fields, but with maximal visual acuity (corrected) of 20/60 to 20/99.

4	Worse eye with marked decrease of fields and maximal visual acuity (corrected) of 20/100 to 20/200; grade 3 plus maximal acuity of better eye of 20/60 or less.
5	Worse eye with maximal visual acuity (corrected) less than 20/200; grade **4** plus maximal acuity of better eye of 20/60 or less.
6	Grade **5** plus maximal visual acuity of better eye of 20/60 or less.
V	Unknown.
X	Is added to grades 0 to 6 for presence of temporal pallor.

7. Cerebral (or Mental) Functions

Score	Description
0	Normal.
1	Mood alteration only (Does not affect DSS score).
2	Mild decrease in mentation.
3	Moderate decrease in mentation.
4	Marked decrease in mentation (chronic brain syndrome – moderate).
5	Dementia or chronic brain syndrome-severe or incompetent.
V	Unknown.

8. Other Functions

Score	Description
0	None.
1	Any other neurologic findings attributed to MS (specify).
V	Unknown.

APPENDIX II

Cyprus National Bioethics Committee

The approval involves the studies described in chapter 2 and chapter 3.

REPUBLIC OF CYPRUS

CYPRUS NATIONAL BIOETHICS COMMITTEE

Ref.: ΕΕΒΚ/ΕΠ /2022/32
Tel.: 22809038 / 22809039
Fax: 22353878

5th of July, 2022

Dr Nikos Konstantinou
Assistant Professor of Cognitive Neuroscience
Department of Rehabilitation Sciences
Cyprus University of Technology
Vragadinou 15
Limassol 3041

Dear Dr Konstantinou,

Research Proposal entitled:
« The investigation of the effects of sport activities in clinical condition of patients with Multiple Sclerosis »

With regards to the above research proposal, we would like to confirm that the bioethical review procedure by the Cyprus National Bioethics Committee (CNBC) has been completed.

2. According to the decision issued by the Review Bioethics Committee (Form EEBK 04) on the 10th of June 2022, which has already been sent to you, the above mentioned research proposal has been approved.

3. We would like to wish you every success in the conduct of your research, and kindly ask you to inform us about any amendments in the research design that may occur after the issuance of the current opinion, as well as provide us with feedback on the progress of the study using the designated forms available on the Committee´s website.

Yours sincerely,

Prof. Constantinos N. Phellas
Chairman
Cyprus National Bioethics Committee

22, Laertou Str., 2365 Agios Dometios, Nicosia
Email: cnbc@bioethics.gov.cy, *Website:* www.bioethics.gov.cy

APPENDIX III

Clinical assessments forms

Mini-Balance Evaluation Systems Test

ID:_____ Date:_____

ANTICIPATORY	***SUB SCORE:*** **/ 6**
1. SIT TO STAND *Instruction: "Cross your arms across your chest. Try not to use your hands unless you must. Do not let your legs lean against the back of the chair when you stand. Please stand up now."* (2) Normal: Comes to stand without use of hands and stabilizes independently. (1) Moderate: Comes to stand WITH use of hands on first attempt. (0) Severe: Unable to stand up from chair without assistance, OR needs several attempts with use of hands.	
2. RISE TO TOES *Instruction: "Place your feet shoulder width apart. Place your hands on your hips. Try to rise as high as you can onto your toes. I will count out loud to 3 seconds. Try to hold this pose for at least 3 seconds. Look straight ahead. Rise now."* **(2) Normal: Stable for 3 s with maximum height.** **(1) Moderate: Heels up, but not full range (smaller than when holding hands), OR noticeable instability for 3s.** (0) Severe: < 3 s.	
3. STAND ON ONE LEG *Instruction: "Look straight ahead. Keep your hands on your hips. Lift your leg off of the ground behind you without touching or resting your raised leg upon your other standing leg. Stay standing on one leg as long as you can. Look straight ahead. Lift now.*	
Left: Time in Seconds **Trial 1:_____Trial 2:_____** **(2) Normal: 20 s.** **(1) Moderate: < 20 s.** (0) Severe: Unable.	**Right: Time in Seconds** **Trial 1:_____Trial 2:_____** (2) Normal: 20 s. (1) Moderate: < 20 s. (0) Severe: Unable.

REACTIVE POSTURAL CONTROL ***SUB SCORE:*** **/ 6**

4. COMPENSATORY STEPPING CORRECTION- FORWARD

Instruction: "Stand with your feet shoulder width apart, arms at your sides. Lean forward against my hands beyond your forward limits. When I let go, do whatever is necessary, including taking a step, to avoid a fall."

(2) Normal: Recovers independently with a single, large step (second realignment step is allowed).

(1) Moderate: More than one step used to recover equilibrium.

(0) Severe: No step, OR would fall if not caught, OR falls spontaneously.

5. COMPENSATORY STEPPING CORRECTION- BACKWARD

Instruction: "Stand with your feet shoulder width apart, arms at your sides. Lean backward against my hands beyond your backward limits. When I let go, do whatever is necessary, including taking a step, to avoid a fall."

(2) Normal: Recovers independently with a single, large step.

(1) Moderate: More than one step used to recover equilibrium.

(0) Severe: No step, OR would fall if not caught, OR falls spontaneously.

6. COMPENSATORY STEPPING CORRECTION- LATERAL

Instruction: "Stand with your feet together, arms down at your sides. Lean into my hand beyond your sideways limit. When I let go, do whatever is necessary, including taking a step, to avoid a fall."

Left	**Right**
(2) Normal: Recovers independently with 1 step (crossover or lateral OK). **(1) Moderate: Several steps to recover equilibrium** (0) Severe: Falls, or cannot step.	(2) Normal: Recovers independently with 1 step (crossover or lateral OK). (1) Moderate: Several steps to recover equilibrium (0) Severe: Falls, or cannot step.

SENSORY ORIENTATION	***SUB SCORE:*** **/ 6**
7. STANCE (FEET TOGETHER); EYES OPEN, FIRM SURFACE *Instruction: "Place your hands on your hips. Place your feet together until almost touching. Look straight ahead. Be as stable and still as possible, until I say stop."* *Time in seconds:_____* **(2) Normal: 30 s.** **(1) Moderate: < 30 s.** (0) Severe: Unable.	
8. STANCE (FEET TOGETHER); EYES CLOSED, FOAM SURFACE *Instruction: "Step onto the foam. Place your hands on your hips. Place your feet together until almost touching. Be as stable and still as possible, until I say stop. I will start timing when you close your eyes."* *Time in seconds:_____* **(2) Normal: 30 s.** **(1) Moderate: < 30 s.** (0) Severe: Unable.	
9. INCLINE- EYES CLOSED *Instruction: "Step onto the incline ramp. Please stand on the incline ramp with your* toes toward the top. Place your feet shoulder width apart and have your arms down at your sides. I will start timing when you close your eyes." Time in seconds:_____ **(2) Normal: Stands independently 30 s and aligns with gravity.** **(1) Moderate: Stands independently <30 s OR aligns with surface.** (0) Severe: Unable.	
DYNAMIC GAIT	***SUB SCORE:*** **/ 6**
10. CHANGE IN GAIT SPEED *Instruction: "Begin walking at your normal speed, when I tell you 'fast', walk as fast as you can. When I say 'slow', walk very slowly."* **(2) Normal: Significantly changes walking speed without imbalance.** **(1) Moderate: Unable to change walking speed or signs of imbalance.**	

(0) Severe: Unable to achieve significant change in walking speed AND signs of imbalance.

11. WALK WITH HEAD TURNS – HORIZONTAL

Instruction: "Begin walking at your normal speed, when I say "right", turn your head and look to the right. When I say "left" turn your head and look to the left. Try to keep yourself walking in a straight line."

(2) Normal: performs head turns with no change in gait speed and good balance.

(1) Moderate: performs head turns with reduction in gait speed.

(0) Severe: performs head turns with imbalance.

12. WALK WITH PIVOT TURNS

Instruction: "Begin walking at your normal speed. When I tell you to 'turn and stop', turn as quickly as you can, face the opposite direction, and stop. After the turn, your feet should be close together."

(2) Normal: Turns with feet close FAST (< 3 steps) with good balance.

(1) Moderate: Turns with feet close SLOW (>4 steps) with good balance.

(0) Severe: Cannot turn with feet close at any speed without imbalance.

13. STEP OVER OBSTACLES

Instruction: "Begin walking at your normal speed. When you get to the box, step over it, not around it and keep walking."

(2) Normal: Able to step over box with minimal change of gait speed and with good balance.

(1) Moderate: Steps over box but touches box OR displays cautious behavior by slowing gait.

(0) Severe: Unable to step over box OR steps around box.

14. TIMED UP & GO WITH DUAL TASK *[3 METER WALK]Instruction*

TUG: - Instruction TUG: "When I say 'Go', stand up from chair, walk at your normal speed across the tape on the floor, turn around, and come back to sit in the chair."

- Instruction TUG with Dual Task: "Count backwards by threes starting at ___. When I say 'Go', stand up from chair, walk at your normal speed across the tape on the floor, turn around, and come back to sit in the chair. Continue counting backwards the entire time."

TUG: _____seconds; Dual Task TUG: _____seconds

(2) Normal: No noticeable change in sitting, standing or walking while backward counting when compared to TUG without Dual Task.

(1) Moderate: Dual Task affects either counting OR walking (>10%) when compared to the TUG without Dual Task.

(0) Severe: Stops counting while walking OR stops walking while counting.

When scoring item 14, if subject's gait speed slows more than 10% between the TUG without and with a Dual Task the score should be decreased by a point.

Instructions

Subject Conditions: Subject should be tested with flat-heeled shoes OR shoes and socks off.

Equipment: Temper® foam (also called T-foam™ 4 inches thick, medium density T41 firmness rating)**,** chair without arm rests or wheels, incline ramp, stopwatch, a box (9" height) and a 3 meter distance measured out and marked on the floor with tape [from chair].

Scoring: The test has a maximum score of **28** points from **14 items** that are each scored from 0-2.

"0" indicates the lowest level of function and "2" the highest level of function.

If a subject must use an assistive device for an item, score that item one category lower.

If a subject requires physical assistance to perform an item, score "0" for that item.

For **Item 3** (stand on one leg) and **Item 6** (compensatory stepping-lateral) only include the score for one side (the worse score).

For **Item 3** (stand on one leg) select the best time of the 2 trials [from a given side] for the score.

For **Item 14** (timed up & go with dual task) if a person's gait slows greater than 10% between the TUG without and with a dual task then the score should be decreased by a point.

1. SIT TO STAND	Note the initiation of the movement, and the use of the subject's hands on the seat of the chair, the thighs, or the thrusting of the arms forward.
2. RISE TO TOES	Allow the subject two attempts. Score the best attempt. (If you suspect that subject is using less than full height, ask the subject to rise up while holding the examiners' hands.) Make sure the subject looks at a non-moving target 4-12 feet away.
3. STAND ON ONE LEG	Allow the subject two attempts and record the times. Record the number of seconds the subject can hold up to a maximum of 20 seconds. Stop timing when the subject moves hands off of hips or puts a foot down. Make sure the subject looks at a non-moving target 4-12 feet ahead. Repeat on other side.
4. COMPENSATORY STEPPING CORRECTION-FORWARD	Stand in front of the subject with one hand on each shoulder and ask the subject to lean forward (Make sure there is room for them to step forward). Require the subject to lean until the subject's shoulders and hips are in front of toes. After you feel the subject's body weight in your hands, very suddenly release your support. The test must elicit a step. NOTE: Be prepared to catch subject.
5. COMPENSATORY STEPPING CORRECTION - BACKWARD	Stand behind the subject with one hand on each scapula and ask the subject to lean backward (Make sure there is room for the subject to step backward.) Require the subject to lean until their shoulders and hips are in back of their heels. After you feel the subject's body weight in your hands, very suddenly release your support. Test must elicit a step. NOTE: Be prepared to catch subject.
6. COMPENSATORY STEPPING CORRECTION-LATERAL	Stand to the side of the subject, place one hand on the side of the subject's pelvis, and have the subject lean their whole body into your hands. Require the subject to lean until the midline of the pelvis is over the right (or left) foot and then

	suddenly release your hold. NOTE: Be prepared to catch subject.
7. STANCE (FEET TOGETHER); EYES OPEN, FIRM SURFACE	Record the time the subject was able to stand with feet together up to a maximum of 30 seconds. Make sure subject looks at a non-moving target 4-12 feet away.
8. STANCE (FEET TOGETHER); EYES CLOSED, FOAM SURFACE	Use medium density Temper® foam, 4 inches thick. Assist subject in stepping onto foam. Record the time the subject was able to stand in each condition to a maximum of 30 seconds. Have the subject step off of the foam between trials. Flip the foam over between each trial to ensure the foam has retained its shape.
9. INCLINE EYES CLOSED	Aid the subject onto the ramp. Once the subject closes eyes, begin timing and record time. Note if there is excessive sway.
10. CHANGE IN SPEED	Allow the subject to take 3-5 steps at normal speed, and then say “fast”. After 3-5 fast steps, say “slow”. Allow 3-5 slow steps before the subject stops walking.
11. WALK WITH HEAD TURNSHORIZONTAL	Allow the subject to reach normal speed, and give the commands “right, left” every 3-5 steps. Score if you see a problem in either direction. If subject has severe cervical restrictions allow combined head and trunk movements.
12. WALK WITH PIVOT TURNS	Allow the subject to reach normal speed, and give the commands “right, left” every 3-5 steps. Score if you see a problem in either direction. If subject has severe cervical restrictions allow combined head and trunk movements.
13. STEP OVER OBSTACLES	Place the box (9 inches or 23 cm height) 10 feet away from where the subject will begin walking. Two shoeboxes taped together works well to create this apparatus.
14. TIMED UP & GO WITH DUAL TASK	Use the TUG time to determine the effects of dual tasking. The subject should walk a 3 meter distance. TUG: Have the subject sitting with the subject’s back against the chair. The

	subject will be timed from the moment you say “Go” until the subject returns to sitting. Stop timing when the subject’s buttocks hit the chair bottom and the subject’s back is against the chair. The chair should be firm without arms. TUG With Dual Task: While sitting determine how fast and accurately the subject can count backwards by threes starting from a number between 100-90. Then, ask the subject to count from a different number and after a few numbers say “Go”. Time the subject from the moment you say “Go” until the subject returns to the sitting position. Score dual task as affecting counting or walking if speed slows (>10%) from TUG and or new signs of imbalance.

Six Spot Step Test

ID: _____ Date: _____ Dominant leg:_____

Dominant Leg	**Non-Dominant Leg**
Trial 1	**Trial 1**
Time:	**Time:**
For a complete trial, record any circumstances that affected the patient's performance _____	For a complete trial, record any circumstances that affected the patient's performance _____
If trial was not completed (mark one): □ Unable to complete trial due to physical limitation □ Other Specify _____	If trial was not completed (mark one): □ Unable to complete trial due to physical limitation □ Other Specify _____

Dominant Leg	**Non-Dominant Leg**
Trial 2	**Trial 2**
Time:	**Time:**
For a complete trial, record any circumstances that affected the patient's performance _____	For a complete trial, record any circumstances that affected the patient's performance _____
If trial was not completed (mark one): □ Unable to complete trial due to physical limitation □ Other Specify _____	If trial was not completed (mark one): □ Unable to complete trial due to physical limitation □ Other Specify _____

Average time of the four runs:……..

Action Research Arm Test

ID: _____ Date: _____

A) Grasp Subscale

Test	Description	Time	Cut off Point left/right	Score Left	Score Right
1	Block 10 cm (If score = 3 total A = 18 and go to subscale B)		4.3 / 4.1 sec		
2	Block 2.5 cm (If score = 0 then total A = 0 and go to B)		3.6 / 3.5 sec		
3	Block 5 cm		3.6 / 3.5 sec		
4	Block 7.5 cm		3.9 / 3.8 sec		
5	Ball (7.5 cm diameter)		3.9 / 3.7 sec		
6	Metal rectangle		3.8 / 3.5 sec		
			Total A:		

B) Grip Subscale

Test	Description	Time	Cut off Point left/right	Score Left	Score Right
1	Pour water from one cup to another (If score = 3 total B = 12 and go to subscale C)		7.9 / 7.8 sec		
2	Displace tube 2.5cm from one side the other (If score = 0 total B = 0 and go to subscale C)		4.2 / 4.1 sec		

3	Displace tube 1cm from one side to the other		4.4 / 4.1 sec		
4	Ring (3.5cm) with synthetic hold		4.1 / 3.9 sec		
			Total B:		

C) <u>Pinch Subscale</u>

Test	Description	Time	Cut off Point left/right	Score Left	Score Right
1	Ball bearing 6mm (thumb - ring finger) (If score = 3 total C = 18 and go to subscale D)		4.5 / 4.4 sec		
2	Marble 1.5cm (thumb - index finger) (If score = 0 total C = 0 and go to subscale D)		3.9 / 3.7 sec		
3	Marble 1.5cm (thumb - middle finger)		3.9 / 3.8 sec		
4	Ball 6mm (thumb - index finger)		4.2 / 3.8 sec		
5	Marble 1.5cm (thumb - ring finger)		4.2 / 3.8 sec		
6	Marble 1.5cm (thumb - middle finger)		4.1 / 4.0 sec		
			Total C:		

D) <u>Gross Movements Subscale</u>

Test	Description	Time	Cut off Point left/right	Score Left	Score Right
1	Hand to behind the head (If score = 3 total C = 9, END test)		2.8 / 2.6 sec		

2	Hand to the top of the head (If score = 0 total C = 0, END test)		2.5 / 2.4 sec		
3	Hand to the mouth		2.8 / 2.6 sec		
			Total D:		

TOTAL SCORE (maximum score = 57) = Left: Right:

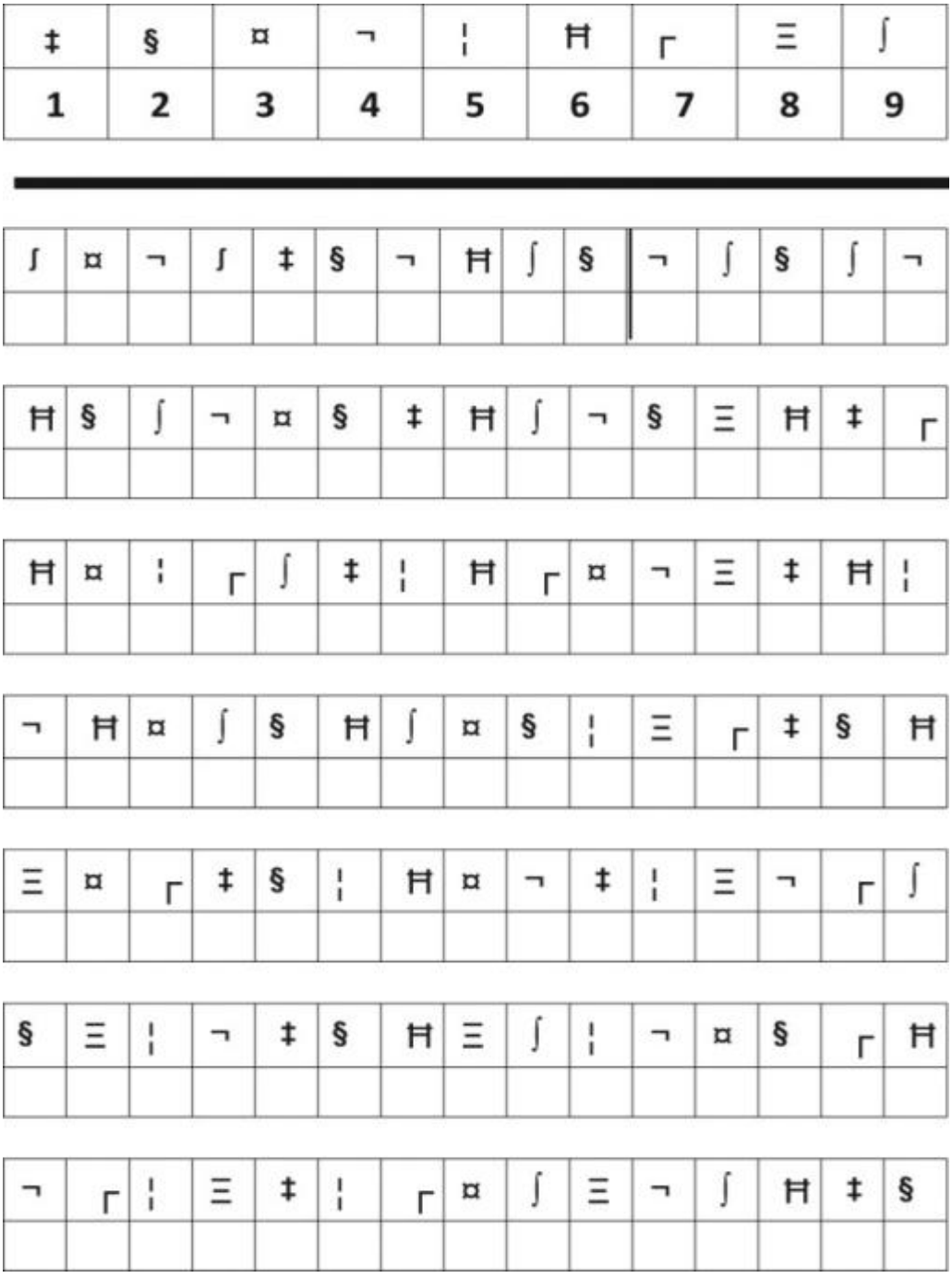
Hand Held Dynamometer

ID: _____ Date: _____ Dominant hand: _____

Muscle Group	Left	Right
Shoulder flexors		
Shoulder extensors		
Shoulder internal rotators		
Shoulder external rotators		
Shoulder adductors		
Shoulder abductors		
Shoulder horizontal adductors		
Shoulder horizontal abductors		
Elbow flexors		
Elbow extensors		

Symbol Digit Modalities Test

ID: _____ Date: _____



Total score:_____

Note: The specific form it is the one which was used for the first assessment in baseline phase. To diminish the practice effect, six different tests were created, as many as our assessment points, in which the key was rearranged.

Purdue Pegboard Test

ID: _____ Date: _____ Dominant hand: _____

Test	Score
Unimanual task - Dominant hand (30s)	
Unimanual task - Non-dominant hand (30s)	
Bimanual task – inphase (30s) (pairs)	
Bimanual – antiphase (60s)	

Norms

Purdue Subtests	Females	Males
Right Hand	24.9 – 0.15 x (age)	22.5 – 0.15 x (age)
Left Hand	23.7 – 0.16 x (age)	24.1 – 0.18 x (age)
Both Hands	19.9 – 0.14 x (age)	20 – 0.15 x (age)
Right + Left + Both Hands	67.7 – 0.45 (age)	66.5 – 0.48 x (age)
Assembly	59.4 – 0.45 x (age)	62.2 – 0.53 x (age)

Example: The expected score for an 80-year-old woman on the right-hand task is: 24.0 – (0.15 x 80) = 12.

Instructions

1. In the first subtest, dominant hand of participant is required to pick up pins one by one from the cup on the dominant hand side and insert them into the corresponding column of holes, starting with the hole farthest away from the participant (e.g., right-handed take pins from right cup and insert them in the right column).

2. In the second subtest, pins picked up from the non-dominant hand side of the cup with the non-dominant hand are inserted into the non-dominant hand side column of holes.

3. The third subtest is a synchronous bimanual task that requires simultaneous use of both hands to grasp pins from their corresponding cups (i.e., right hand-right cup, left hand-left cup) and place them in their corresponding columns of holes.

4. The fourth subtest involves alternating movements of both hands to complete assemblies of different types of pegs including pins, washers, and collars, in the right column of holes.

Scoring

Standard scoring of the Purdue Pegboard Test is based on the number of pegs inserted in 30 second for the first three subtests and in 60 seconds for the fourth subtest.

Timed 25-Foot Walk

ID: _____ Date: _____

Did patient wear an AFO?	□ Yes		□ No
Was assistive device used?	□ Yes		□ No
Assistive device used (*mark one*):			
□ Unilateral device	□ Cane	□ Crutch	
□ Bilateral Assistance	□ Cane	□ Crutch	□ Walker/Rollator

Trial 1

Time for 25-Foot Walk (sec) : _____

For a complete trial, record any circumstances that affected the patient's performance:

_____

_____

If trial was not completed (*mark one*): Specify

□ Unable to complete trial due to physical limitations:_____

□ Other:_____

Trial 2

Time for 25-Foot Walk (sec) : _____

For a complete trial, record any circumstances that affected the patient's performance:

_____

_____

If trial was not completed (*mark one*): Specify

□ Unable to complete trial due to physical limitations:_____

□ Other:_____

Did it take more than two attempts to get two successful trials? □ Yes □ No

If yes, please specify reason(s) for more than two attempted trials:

_____

Modified Fatigue Impact Scale

ID: _____ Date: _____

Below is a list of statements that describe the effects of fatigue during the past four weeks. Please read each statement carefully and circle the number which indicates how often fatigue has affected you in this way. You have to answer every question, but if you are not sure about an answer, select the one that comes closest to your description.

Because of my fatigue during the past 4 weeks

No.	Statement	Never	Rarely	Sometimes	Often	Almost Always
1	I have been less alert	0	1	2	3	4
2	I have had difficulty paying attention for long periods of time.	0	1	2	3	4
3	I have been unable to think clearly.	0	1	2	3	4
4	I have been clumsy and uncoordinated.	0	1	2	3	4
5	I have been forgetful.	0	1	2	3	4
6	I have had to pace myself in my physical activities.	0	1	2	3	4
7	I have been less motivated to do anything that requires physical effort.	0	1	2	3	4
8	I have been less motivated to participate in social activities.	0	1	2	3	4

9	I have been limited in my ability to do things away from home.	0	1	2	3	4
10	I have trouble maintaining physical effort for long periods.	0	1	2	3	4
11	I have had difficulty making decisions.	0	1	2	3	4
12	I have been less motivated to do anything that requires thinking.	0	1	2	3	4
13	My muscles have felt weak.	0	1	2	3	4
14	I have been physically uncomfortable.	0	1	2	3	4
15	I have had trouble finishing tasks that require thinking.	0	1	2	3	4
16	I have had difficulty organizing my thoughts when doing things at home or at work.	0	1	2	3	4
17	I have been less able to complete tasks that require physical effort.	0	1	2	3	4
18	My thinking has been slowed down.	0	1	2	3	4
19	I have had trouble concentrating.	0	1	2	3	4
20	I have limited my physical activities.	0	1	2	3	4

21	I have needed to rest more often or for longer periods.	0	1	2	3	4

Instructions for Scoring

All items can be aggregated into three subscales (i.e., physical, cognitive, and psychosocial), as well as into a total score. The higher the score is, the greater is the impact of fatigue on a participant's activities.

1. Physical Subscale

This scale can range from 0 to 36. It is calculated by adding the scores of the following items: 4+6+7+10+13+14+17+20+21 = ……

2. Cognitive Subscale

This scale can range from 0 to 40. It is calculated by adding the scores of the following items: 1+2+3+5+11+12+15+16+18+19 = ……

3. Psychosocial Subscale

This scale can range from 0 to 8. It is calculated by adding the scores of the following items: 8+9 = ……

Total Score

The total score can range from 0 to 84. It is calculated by adding the scores of the physical, cognitive, and psychosocial subscales.

Total score = ……

Medical Outcomes Study Questionnaire Short Form 36 Health Survey

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. Thank you for completing this survey! For each of the following questions, please circle the number that best describes your answer.

1. In general, would you say your health is:	
Excellent	1
Very good	2
Good	3
Fair	4
Poor	5

2. Compared to one year ago:	
Much better now than one year ago	1
Somewhat better now than one year ago	2
About the same	3
Somewhat worse now than one year ago	4
Much worse now than one year ago	5

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

(Circle One Number on Each Line)

	Yes, Limited a Lot (1)	**Yes, Limited a Little (2)**	**No, Not limited at All (3)**
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
c. Lifting or carrying groceries	1	2	3
d. Climbing several flights of stairs	1	2	3
e. Climbing one flight of stairs	1	2	3
f. Bending, kneeling, or stooping	1	2	3
g. Walking more than a mile	1	2	3
h. Walking several blocks	1	2	3
i. Walking one block	1	2	3
j. Bathing or dressing yourself	1	2	3

4. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

(Circle One Number on Each Line)

	Yes (1)	No (2)
a. Cut down the amount of time you spent on work or other activities	1	2
b. Accomplished less than you would like	1	2
c. Were limited in the kind of work or other activities	1	2
d. Had difficulty performing the work or other activities (for example, it took extra effort)	1	2

5. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

(Circle One Number on Each Line)

	Yes (1)	No (2)
a. Cut down the amount of time you spent on work or other activities	1	2
b. Accomplished less than you would like	1	2
c. Didn't do work or other activities as carefully as usual	1	2

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?	
Not at all	1
Slightly	2
Moderately	3
Quite a bit	4
Extremely	5
7. How much bodily pain have you had during the past 4 weeks?	
None	1
Very mild	2
Mild	3
Moderate	4
Severe	5
Very severe	6
8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?	
Not at all	1
A little bit	2
Moderately	3
Quite a bit	4
Extremely	5

These questions are about how you feel and how things have been with you **during the past 4 weeks.** For each question, please give the one answer that comes closest to the way you have been feeling.

(Circle One Number on Each Line)

9. How much of the time during the past 4 weeks……

	All of the Time	**Most of the Time**	**A Good Bit of the Time**	**Some of the Time**	**A Little of the Time**	**None of the Time**
a. Did you feel full of pep?	1	2	3	4	5	6
b. Have you been a very nervous person?	1	2	3	4	5	6
c. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
d. Have you felt calm and peaceful?	1	2	3	4	5	6
e. Did you have a lot of energy?	1	2	3	4	5	6
f. Have you felt downhearted and blue?	1	2	3	4	5	6
g. Did you feel worn out?	1	2	3	4	5	6
h. Have you been a happy person?	1	2	3	4	5	6
i. Did you feel tired?	1	2	3	4	5	6

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)? (Circle One Number)	
All of the time	1
Most of the time	2
Some of the time	3
A little of the time	4
None of the time	5

11. How **TRUE** or **FALSE** is each of the following statements for you.

(Circle One Number on Each Line)

	Definitely True	**Mostly True**	**Don't Know**	**Mostly False**	**Definitely False**
a. I seem to get sick a little easier than other people	1	2	3	4	5
b. I am as healthy as anybody I know	1	2	3	4	5
c. I expect my health to get worse	1	2	3	4	5
d. My health is excellent	1	2	3	4	5

APPENDIX IV

Examples of the exercise protocol.

Figure 1. Basketball chest pass.

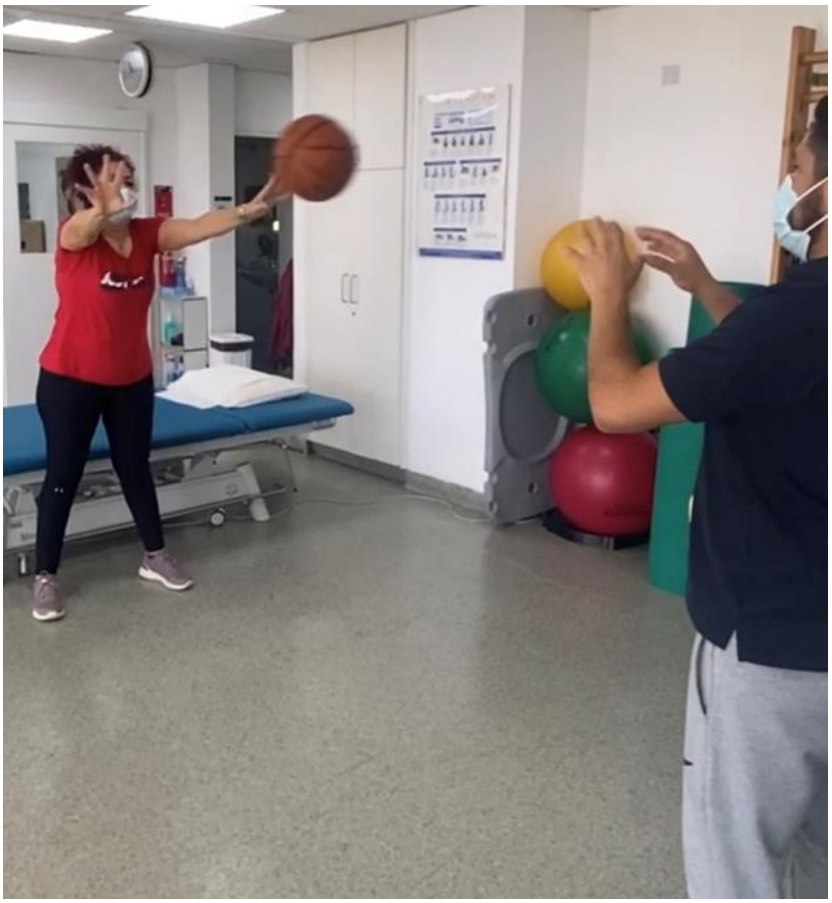


Figure 1: The basketball chest pass is named so because the pass originates from the chest. During the exercise both hands have contact with the ball and thumbs are directly placed behind the ball. The contact with ball should be made at the chest level, with feet and body being behind the ball and feet (base) should be comfortable and solid. As contact is made, participants should push the ball to the trainer by extending both arms out from the chest.

Figure 2. Basketball shoulder pass.

Figure 2: The basketball shoulder pass it is known as also as an overhead pass. During the exercise both hands are placed on the side of the ball. The contact with ball should be made over the participants' head, and feet (base) should be comfortable and solid. As contact is made, participants should bring the ball behind the head by flexing both elbows and shoulders and then push the ball to the trainer by extending both shoulders and elbows over the head with the ball and throw forward targeting trainer's head.

Figure 3. Volleyball overhead pass.

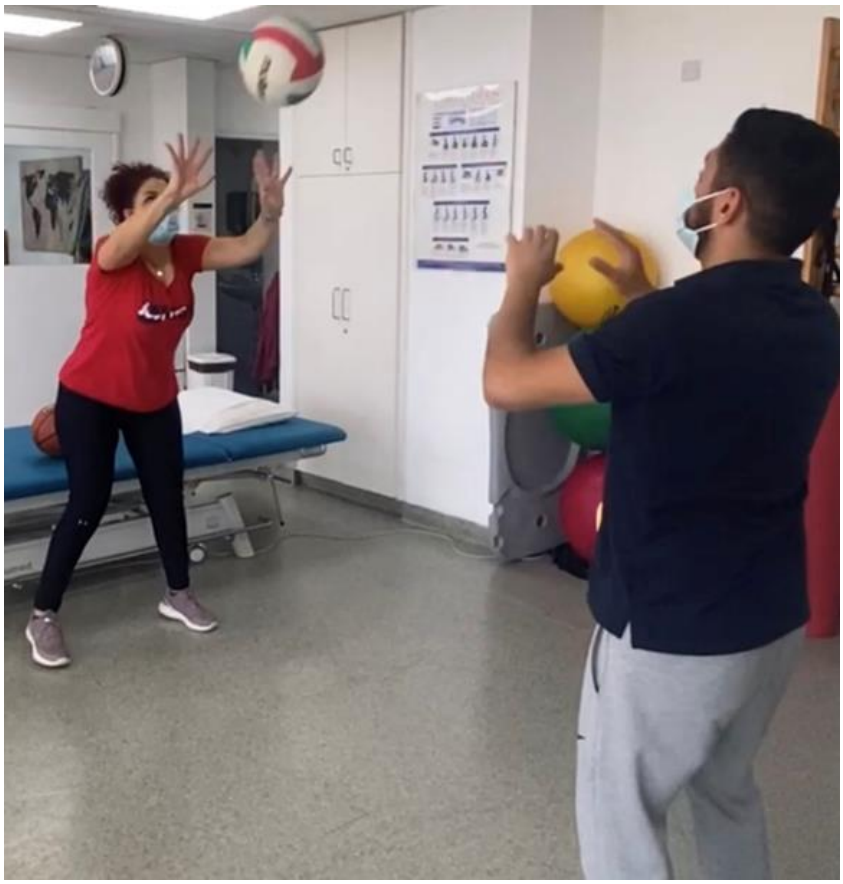


Figure 3: During the volleyball overhead pass, fingers must be spread and firm, both hands in front of face. The contact with ball should be made at a level in front of the face. Feet and body must be behind the ball, feet (base) should be comfortable and solid. Hands and fingers should remain firm and strong on contact. As contact is made, participants should push the ball to the trainer by extending both arms out from the face.

Figure 4. Proprioceptive Neuromuscular Facilitation exercises (PNF).

Figure 4: a) PNF 1st diagonal flexion pattern, b) PNF 1st diagonal extension pattern, c) PNF 2nd diagonal flexion pattern, d) a) PNF 2nd diagonal extension pattern. Participants follow the exercise protocol, they performed bilateral upper limbs PNF patterns. All details regarding upper limbs movements and muscle groups activation are presented in table 1 The level of difficulty, was managed over the color of the elastic bands (i.e., red color = small resistance, green color = medium resistance)

Table 1. Proprioceptive Neuromuscular Facilitation (PNF) – Diagonals – Joint movements.

Unilateral Upper Limb PNF patterns				
Joint	**1st diagonal flexion pattern**	**1st diagonal extension pattern**	**2nd diagonal flexion pattern**	**2nd diagonal extension pattern**
Shoulder	Flexion Adduction External rotation	Extension Abduction Internal rotation	Flexion Abduction External rotation	Extension Adduction Internal rotation
Elbow	Flexion	Extension	Flexion	Extension
Radioulnar	Supination	Pronation	Supination	Pronation
Wrist	Flexion Adduction	Extension Abduction	Extension Adduction	Flexion Abduction

Fingers	Flexion	Extension	Extension	Flexion
Muscle groups activation	Shoulder joint Shoulder flexors Adductors External rotators. Elbow joint Elbow flexors Radioulnar joint Supinators Wrist and fingers joint Wrist and fingers flexors	Shoulder joint Shoulder extensors Abductors Internal rotators Elbow joint Elbow extensors Radioulnar joint Pronators Wrist and fingers joint Wrist and fingers extensors	Shoulder joint Shoulder flexors Abductors External rotators Elbow joint Elbow flexors Radioulnar joint Supinators Wrist and fingers joint Wrist and fingers extensors	Shoulder joint Shoulder extensors Adductors Internal rotators Elbow joint Elbow extensors Radioulnar joint Pronators Wrist and fingers joint Wrist and fingers flexors

Figure 4. Squat.

Figure 4: Participants must stand with feet shoulder-width apart and toes facing front. During the squats core muscles has to be activated and participants must drive both hips back, bend at the knees and ankles and sit down into a squat position, keeping the heels and toes on the ground, chest up and shoulders back. To lift back to a standing position, they have to press into their heels, engage the gluteal muscles and extend both knees and hips joints. To increase the level of difficulty, balance pads were placed under both feet to provide an unstable base.

Figure 6. Flexion of all fingers.

Figure 6: Participants were seated in an upright position, with both hands placed in an electric bed (height of the bed adjusted at the level of the elbows). During the exercise all fingers of both hands were placed apart in the hand training net, so to perform flexion movement simultaneously against the resistance of the net. The level of difficulty, was managed over the color of the elastic bands (i.e., red color = medium resistance).

Figure 7. Extension of all fingers.

Figure 7: Participants were seated in an upright position, with both hands placed in an electric bed (height of the bed adjusted at the level of the elbows). During the exercise all fingers of both hands were placed close to each other in the hand training net, so to perform extension movement simultaneously against the resistance of the net. The level of difficulty, was managed over the color of the elastic bands (i.e., red color = medium resistance).

Figure 8. Abductors squeeze.

Figure 8: Participants were supine lying in an electric adjustable bed. Both hips and knees were in a flexed position with feet shoulder-width apart and toes facing front. A Pilates ring was placed in between the knees so the participants to perform adduction movement against the resistance of the ring.

Figure 9. Hips Abduction.

Figure 9: Participants were supine lying in an electric adjustable bed. Both hips and knees were in a flexed position with feet shoulder-width apart and toes facing front. Both knees were placed into a Pilates ring so the participants to perform abduction movement against the resistance of the ring.

APPENDIX V

Single Case Pilot Study Design – Results.

Table 1. Individual Central Motor Conduction Time data across all assessment points during baseline and intervention phases – single case pilot study.

Left APB						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
CMCT(ms)	9.2	2.4	7.8	7.9	8.6	7.5
Right APB						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
CMCT(ms)	10	8.8	9.5	8.1	3.7	6.7

Table 1: APB; Abductor Pollicis Brevis, CMCT; Central Motor Conduction Time. Individual data of CMCT (ms) for each phase are presented in Table 1. Data of the participant are presented regarding left and right upper limb, in terms of left and right APB.

Table 2. Individual Motor Evoked Potentials Amplitude data across all assessment points during baseline and intervention phases – single case pilot study.

Left APB						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
MEPs amplitude(mV)	0.11	0.04	0.25	0.73	0.37	0.31
Right APB						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
MEPs amplitude(mV)	0.45	0.46	0.03	0.33	0.09	0.21

Table 2: APB; Abductor Pollicis Brevis, MEPs; Motor Evoked Potentials. Individual data of MEPs amplitude (mV) for each phase are presented in Table 2. Data of the participant are presented regarding left and right upper limb, in terms of left and right APB.

Table 3. Individual Motor Evoked Potentials Latency data across all assessment points during baseline and intervention phases – single case pilot study.

Left APB						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
MEPs latency (msec)	22.3	15.4	20.9	20.9	19.7	20.9
Right APB						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
MEPs latency (msec)	23.2	21.9	22.6	21.2	16.8	21.6

Table 3: APB; Abductor Pollicis Brevis, MEPs; Motor Evoked Potentials. Individual data of MEPs latency (msec) for each phase are presented in Table 3. Data of the participant are presented regarding left and right upper limb, in terms of left and right APB.

Table 4. Individual Resting Motor Threshold data across all assessment points during baseline and intervention phases – single case pilot study.

Left APB						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
rMT (%MSO)	50	49	45	47	47	47
Right APB						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
rMT (%MSO)	50	48	48	44	49	48

Table 4: APB; Abductor Pollicis Brevis, rMT; resting motor threshold, MSO; Maximum Stimulator Output. Individual data of rMT (%MSO) for each phase are presented in Table 4. Data of the participant are presented regarding left and right upper limb, in terms of left and right APB.

Table 5. Individual Mini Balance Evaluation System Test data across all assessment points during baseline and intervention phases – single case pilot study.

Mini Balance Evaluation System Test						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
Score	26	27	28	28	28	27.7

Table 5: Individual data of Mini Balance Evaluation System Test for each phase are presented in Table 5.

Table 6. Individual Six Spot Step Test data across all assessment points during baseline and intervention phases – single case pilot study.

Six Spot Step Test						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
Score (sec)	12.5	8.1	6.9	6.8	6.5	7

Table 6: Individual data of Six Spot Step Test for each phase are presented in Table 6.

Table 7. Individual Action Research Arm Test data across all assessment points during baseline and intervention phases – single case pilot study.

Left Upper Limb						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
Score	53	57	57	57	57	57
Right Upper Limb						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
Score	50	57	57	57	57	57

Table 7: Individual data of Action Research Arm Test for each phase are presented in Table 7.

Table 8. Individual data of the Muscle Strength Tests across all assessment points during baseline and intervention phases – single case pilot study.

Study Phases	**Baseline**	**Intervention**				**Mean Intervention**
Hand Grip						
Left side Score(kg)	8.1	8.8	10.6	11.1	13.4	10.9
Right side Score(kg)	13.3	13.3	13.4	13.4	13.6	13.4
Shoulder Flexors						
Left side Score(kg)	11.1	11.2	12.8	12.8	14.4	12.7
Right side Score(kg)	8.9	12.3	12	13.7	14	13
Shoulder Extensors						
Left side Score(kg)	11.1	12.4	13.9	14	14.6	13.7
Right side Score(kg)	8.8	11.3	13.9	15.1	16.4	14.1
Shoulder Internal Rotators						
Left side Score(kg)	6	8.5	11.4	11.4	11.4	10.6
Right side Score(kg)	8.1	8.6	10.5	10.9	12.1	10.5
Shoulder External Rotators						

Left side Score(kg)	8.1	8.1	8.3	9.3	10.4	9
Right side Score(kg)	5.3	6.1	7.6	7.9	8.6	7.5
Shoulder Adductors						
Left side Score(kg)	9.6	10.3	13.4	14.6	16.3	13.6
Right side Score(kg)	10.2	12.4	13.5	15.6	20.2	15.4
Shoulder Abductors						
Left side Score(kg)	10.6	11.5	11.9	11.1	14.8	12.3
Right side Score(kg)	7.8	8.4	10.1	10.2	11.8	10
Shoulder Horizontal Adductors						
Left side Score(kg)	7.1	11.1	8.3	10	11.6	10.2
Right side Score(kg)	7.8	8.1	10.6	9.8	10.6	9.7
Shoulder Horizontal Abductors						
Left side Score(kg)	8.1	8.8	9.1	9.6	11.4	9.7
Right side Score(kg)	8	8.2	9.8	9.2	9.6	9.2
Elbow Flexors						
Left side Score(kg)	12.3	12.8	12.9	14.9	15.8	14.1

Right side Score(kg)	9.5	9.9	14.5	16.6	15.8	14.2
Elbow Extensors						
Left side Score(kg)	11.5	12	12.4	13.7	15.1	14.1
Right side Score(kg)	9	14	14.11	14.2	13.8	14

Table 8: Individual data of the Muscle Strength Tests (kg) for each phase are presented in Table 8. The participant showed an improvement for both left and right upper limbs, during the intervention phase.

Table 9. Individual Symbol Digit Modalities Test data across all assessment points during baseline and intervention phases – single case pilot study.

Symbol Digit Modalities Test						
Phases	**Baseline**	**Intervention**				**Mean Intervention**
Score	74	82	100	1005	105	102.5

Table 9: Individual data of Symbol Digit Modalities Test for each phase are presented in Table 9.

Table 10. Individual Modified Fatigue Impact Scale data across all assessment points during baseline and intervention phases – single case pilot study.

Modified Fatigue Impact Scale		
Phases	**Baseline**	**Intervention**
Score	15	16

Table 10: Individual data of Modified Fatigue Impact Scale for each phase are presented in Table 10.

Table 11. Individual the Medical Outcomes Study 36-items Short Form Health Survey data across all assessment points during baseline and intervention phases – single case pilot study.

Medical Outcomes Study 36-items Short Form Health Survey

Phases	Baseline	Intervention
Score	85	91

Table 11: Individual data of Medical Outcomes Study 36-items Short Form Health Survey for each phase are presented in Table 11.

APPENDIX VI_

“ClinicalTrials.gov” related forms

Approval letter

ClinicalTrials.gov Identifier: NCT05367947

Cyprus University of Technology Protocol Record IBEMS,
In-phase Bilateral Exercises in People With Relapsing Remitting Multiple Sclerosis,
is registered and will be posted on the ClinicalTrials.gov public website.

RECORDS USUALLY APPEAR ON ClinicalTrials.gov WITHIN 2 BUSINESS DAYS of the receipt of this message.

Reminder: Review Board approval is required by the time patient recruitment begins. Update the Review Board information in this record when approval has been granted.

QUESTIONS? Contact us at: register@clinicaltrials.gov

Thank you,

PRS Team
ClinicalTrials.gov

SPIRIT Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Investigation of in-phase bilateral exercise effects on corticospinal plasticity in relapsing remitting multiple sclerosis: a registered report single-case concurrent multiple baseline design across five subjects. NCT05367947
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry ClinicalTrials.gov NCT05367947
	2b	All items from the World Health Organization Trial Registration Data Set

- Primary registry and trial identifying number: ClinicalTrials.gov NCT05367947

- Date of registration in primary registry: 10 May, 2022

- Secondary Identifying numbers: N/A

- Source(s) of monetary or material support: Cyprus University of Technology

- Primary sponsor: Cyprus University of Technology

- Secondary sponsor(s): The Cyprus Institute of Neurology and Genetics, The Cyprus Foundation for Muscular Dystrophy Research

-Contact for public queries: DS; sokratous.physio@gmail.com; Cyprus University of Technology; Vragadinou 15, Limassol, 3041; 00357 25002294

- Contact for Scientific Queries: DS, NK, Cyprus University of Technology.

- Public title: In-phase Bilateral Exercises in People With Relapsing Remitting Multiple Sclerosis

- Scientific title: Investigation of in-phase bilateral exercise effects on corticospinal plasticity in relapsing-remitting multiple sclerosis

- Countries of recruitment: Cyprus

- Health condition(s) or problem(s) studied: exercise, clinical condition, corticospinal plasticity

		- Intervention(s): In-phase bilateral exercise of the upper limbs in five people with relapsing remitting multiple sclerosis. - Key Inclusion and Exclusion Criteria: Ages eligible for study: 30-70 years; Sexes eligible for study: both Inclusion criteria: Patients with relapsing-remitting multiple sclerosis, Expanded Disability Status Scale score between three and five. Exclusion criteria: Metal implants, history of cardiovascular or any disease affecting the central nervous system other than multiple sclerosis, pregnancy, epileptic seizures, Spasticity level on upper or lower limbs more than 1+ (slight increase in muscle tone) according to Modified Ashworth Scale.
Protocol version	3	Date and version identifier Issue date: 10 May, 2022 Authors: D.S., C.C.C., E.Z.P., K.M. and N.K.
Funding	4	Sources and types of financial, material, and other support Equipment, consumables and diagnostic devices have been provided by The Cyprus Institute of Neurology and Genetics.

Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors

D.S. [Department of Rehabilitation Sciences, Faculty of Health Sciences, Cyprus University of Technology, Limassol, Cyprus;

Physiotherapy Unit, Neurology Clinics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus].

C.C.C. [Centre for Neuroscience and Integrative Brain Research (CENIBRE), University of Nicosia Medical School, Nicosia, Cyprus].

E.Z.P. [Neuroepidemiology Department, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus].

N.K. [Department of Rehabilitation Sciences, Faculty of Health Sciences, Cyprus University of Technology, Limassol, Cyprus].

K.M. [Biostatistics Unit, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus].

Author contribution

D.S., C.C.C., E.Z.P., and N.K., are responsible for the conception and the experimental design.

D.S., and K.M., are responsible to collect, analyse and interpret the data.

D.S., and N.K., are responsible to draft the manuscript.

N.K., C.C.C., and K.M., revised the manuscript critically for important intellectual content.

5b Name and contact information for the trial sponsor

Investigator Name: Mr. Dimitris Sokratous

Investigator Official Title: MSc

Investigator Affiliation: Cyprus University of Technology, Department of Rehabilitation Sciences, Cyprus University of Technology. Vragadinou 15, Limassol, 3041, 00357 25002294

5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities

N/A

5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)

Principal investigator (PI)

Design and revision of the protocol

Organising steering committee meetings

Managing CTO [clinical trials office]

Publication of study reports

Members of TMC [Trial Management Committee]

Steering committee

(PI, research consultant, senior neurologist, senior physiotherapist)

Agreement of final protocol

All lead investigators will be steering committee members.

Recruitment of patients and liaising with principle [sic] investigator

Reviewing progress of study and if necessary agreeing changes to the protocol and/or facilitate the smooth running of the study.

Trial management committee

(PI, research physician, senior physiotherapist, administrator)

Study planning

Organisation of steering committee meetings

Responsible for trial master file

Advice for lead investigators

Data verification

Randomisation

Data manager committee

(senior physiotherapist, biostastician)

Maintenance of trial IT system and data entry

Data verification

Analysis plan

Data collection and completion

Lead investigators

Senior neurologist and senior physiotherapist are responsible for identification, recruitment.

Lead investigators will be steering committee members.

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention

Introduction: Relapsing Remitting MS (RRMS) is the most common type of MS which is characterised by periods of relapses, that generate unexpected motor symptoms in both body sides.

Mechanism: Motor symptoms are associated with the corticospinal tract integrity, which is quantified by means of corticospinal plasticity which can be probed via transcranial magnetic stimulation (TMS). Several factors, such as exercise and interlimb coordination, can influence corticospinal plasticity.

Existing knowledge: Previous work in healthy and in stroke patients showed that the greatest improvement in corticospinal plasticity occurred during in-bilateral arm exercises. Despite the broad literature on the effects of different types of exercises on the neuroplasticity in people with RRMS, it is unclear whether in-phase bilateral exercises can promote motor related neuroplastic changes in people with MS (pwMS).

Need for a trial: In light of evidence that pwMS have bilateral cortical lesions which cause bilateral changes of corticospinal tract integrity, these findings raise the question about the effects of bilateral exercises on corticospinal plasticity. Such effects would provide strong evidence about whether exercise, in particular in-phase bilateral exercise, can influence the corticospinal plasticity in people with RRMS.

	6b	Explanation for choice of comparators

According to the MS guidelines, various types of exercises are recommended for promoting neuroplasticity and improvement of clinical symptoms. Therefore, a within, as well as a between cases analysis and comparison can be used to identify the possible effects. Alteration in corticospinal plasticity and changes in clinical symptoms of the participants during the three experimental phases (i.e., baseline, intervention, follow-up) encourageed the effectiveness of the proposed intervention.

Objectives	7	Specific objectives or hypotheses

7.1. Research hypothesis

In-phase bilateral exercises of the upper limbs, improve corticospinal plasticity and clinical condition of people with RRMS.

7.2. Primary objectives

To determine if the exercises which include the two types (i.e., in-phase; anti-phase) of in-phase bilateral movement for the upper limbs can promote corticospinal plasticity of people with RRMS.

7.3. Secondary objectives

To determine if the exercises which include the two types (i.e., in-phase; anti-phase) of in-phase bilateral movement for the upper limbs can improve the clinical condition (i.e., motor skills; cognitive functions) of people with RRMS.

Trial design	8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory) The study follows a concurrent multiple baseline design across subjects, without blinding and has been designed according to the 'What Works Clearinghouse' criteria for single case studies. According to Kratochwill et al. (2010), three participants, with collection of three data points for each across different phases is the minimum number needed to meet the standard criteria, while four or more is recognized as more reliable. Therefore, we aimed to include five participants to ensure the reliability of the results in case of dropouts, as well as to record several data points across the baseline phase, five data points during the intervention phase and three data points in the follow up phase. However, a randomization of the order it was used, in which the participants were allocated. During the experimental procedure, all participants began the study with the baseline phase at the same time while the intervention phase was introduced staggered across patients and time. The intervention has been introduced systematically in one patient while baseline data collection continues in the others without any intervention.

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained The entire study, including intervention and assessment of outcome measures conducted in the neurophysiology lab and in the physiotherapy unit of The Cyprus Institute of Neurology and Genetics.
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) All participants recruited and evaluated by the senior neurologist of The Cyprus Institute of Neurology and Genetics. Moreover, all participants read and signed a written informed consent while all procedures were approved and conducted in accordance with the ethical guidelines of the Cyprus National Bioethics Committee before recruitment (see Appendix 1_Informed Consent Form). *10.1. Inclusion criteria* 1) diagnosed with RRMS, 2) Expanded Disability Status Scale score between three and five, 3) aged between 30 and 70 years,

4) no relapse within 30 days,

5) Mini Mental State Examination score between 24 and 30 (no cognitive impairment).

10.2. Exclusion criteria

1) metal implants,

2) history of any disease affecting the central nervous system other than MS,

3) history of cardiovascular disease,

4) mental disorders,

5) severe orthopaedic disorders,

6) pregnancy,

7) visual deficit,

8) hearing impairments,

9) epileptic seizures,

10) spasticity level on upper or lower limbs more than 1+ (slight increase in muscle tone) according to Modified Ashworth Scale.

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered

According to the study design (i.e., multiple baseline design across subjects, the intervention was introduced systematically in one patient while baseline data collection continues in the others without any intervention. During the experimental procedure, all participants began the study with the baseline phase at the same time while the intervention phase was introduced staggered across patients and time. Therefore, five people with RRMS were allocated randomly. The intervention protocol lasted for 12 consecutive weeks (30-60 minutes /session x 3 sessions/week) and included in-phase bilateral movements of the upper limbs, adapted to different sports activities and to functional training. Specifically, each session consisted of one to three sets, consisting of 20–30 repetitions of 9 different exercises targeting large muscle groups of the upper limbs (shoulder flexors, extensors, rotators, abductors and adductors, elbow flexors and extensors, hand and finger flexors and extensors). Additionally, three exercises targeted large lower limb muscle groups (hip flexors, extensors, abductors and adductors, knee and ankle flexors and extensors) to be performed in between the upper limbs exercises to allow relaxation of the upper limb muscles.

The specific exercises included sports activities of basic technical skills of basketball (e.g., different types of passing, catching and throwing the ball) and volleyball (e.g., different types of passing and receiving the ball), whereas the fitness exercises included the diagonal movements from proprioceptive neuromuscular facilitation technique, as well as fingers flexion and extension by the use of a resistance hand training net. To maintain the

interest of the participants, the exercise program was modified throughout the course of the 12-week intervention period via changing the level of difficulty. For example, elastic bands with different resistance levels were used and also the number of repetitions and sets varied. Every intervention session consisted of a five minutes' warm-up (i.e., whole body range of motion exercises), followed by the main sport activities and fitness exercise protocol as described above, and a cool down session for five minutes (i.e., passive stretching exercises of the muscle groups which are involved in the main part).

11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)

Fatigue

In order to maintain individual fatigue, each exercise protocol was adapted to the individual needs and with sufficient resting time, as well as with continues monitoring.

However, each participant had to complete at least 27 (75%) out of 36 sessions in order to be included in the data analysis.

11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)

Adherence information sessions

Face-to-face adherence information sessions for all participants took part before starting the experimental procedures. These sessions included:

- The importance of following study guidelines and instructions about the type of exercises and the specific assessment procedures.

- Importance of calling nurses or/and doctors if experiencing any problems or symptoms possibly related to the study.

- There was a brief discussion of reasons for feeling any unexpected symptoms (e.g., pain, fatigue).

Moreover, participants had the opportunity to ask questions from the initial session and reviewed as needed.

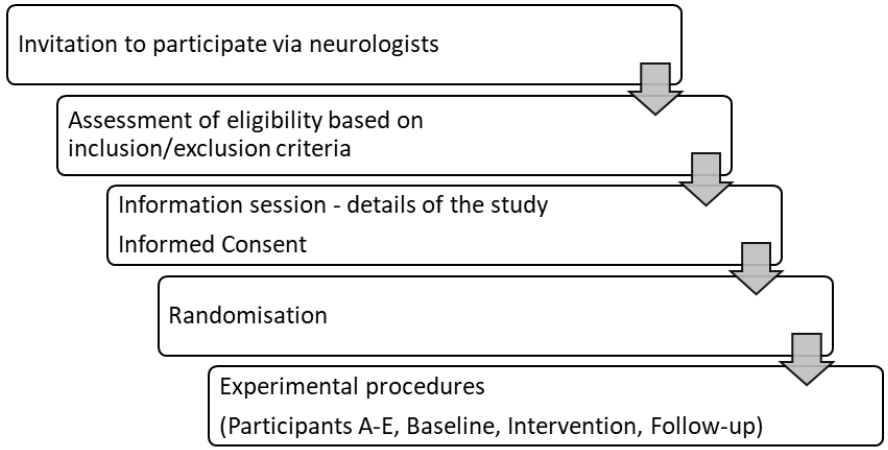
Adherence assessments

To enhance validity of data, an individual electronic data form was used to record all neurophysiological and clinical assessments, which was stored in a secure study computer

	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial Participants were advised to continue their usual prescribed medication throughout the study duration, and they were advised to continue their usual daily routine avoiding receiving any other exercise program during the study.
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended **Primary Outcome Measures** Using electromyography (EMG) signals, we analysed bilateral cortical excitability and bilateral central motor conduction time to determine corticospinal plasticity and therefore to test the primary hypothesis. **Secondary Outcome Measures** We investigated the effects of the specific exercises protocol on the resting motor threshold, on the motor evoke potential (MEP) amplitude and latency of Abductor Pollicis Brevis muscle, which defined cortical excitability. Clinical symptoms using clinical assessment (i.e., gait, balance, strength, hand dexterity, cognitive functions) Mini Balance Evaluation Systems Test measures dynamic balance, functional mobility, and gait in neurological

patients, including people with RRMS, the Six Spot Step Test is an assessment tool that evaluates a complex range of sensorimotor functions, part of which are lower limb strength, spasticity, coordination, as well as balance. We well assess the isometric muscle force of major muscle groups with the use of the muscle controller (Kinvent Biomechanique, Montpelier, France) which is a dynamometer used in the evaluation and rehabilitation of muscle strength that provides real time biofeedback. Also, we employed the oral form of the Symbol Digit Modalities Test which assesses the information processing speed. Finally, we performed the Modified Fatigue Impact Scale which is a short questionnaire that requires the participants to describe the effects of fatigue during the past four weeks and the Medical Outcomes Study Questionnaire Short Form 36 Health Survey. This is a set of generic, coherent, and easily administered quality-of-life subjective questionnaire.

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)



For the experimental procedures see also Appendix 3_ Figure 1.

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations

The study follows a concurrent multiple baseline design across subjects, without blinding and has been designed according to the 'What Works Clearinghouse' criteria for single case studies. According to Kratochwill et al. (2010), three participants, with collection of three data points across different phases is the

minimum number needed to meet the standard criteria, while four or more is recognized as more reliable. We aimed to include five participants to ensure the reliability of the results in case of dropouts, as well as to record several data points across the baseline phase, five data points during the intervention phase and three data points in the follow up phase. During the experimental procedure, all participants began the study with the baseline phase at the same time while the intervention phase is introduced staggered across patients and time (see Appendix 2). The intervention introduced systematically in one patient while baseline data collection continues in the others without any intervention. The cause-effect inference can be clearly verified by the staggered duration through separate baseline phases. Subsequently, if the intervention (i.e., in-phase bilateral exercises) was the sole cause of improvement in participants' conditions, the proposed outcome measures did not change for the participants that were remained in the baseline phase but improved only for those in the intervention phase.

Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

One of the affiliated organizations was the Cyprus Institute of Neurology and Genetics which is a medical and biomedical translation centre, promoting patient care, research and educational programs on neurological disease, including MS. Therefore, patients were recruited mainly from the senior neurologist of the Cyprus Institute of Neurology and Genetics throughout the patient registry (database). Once identified in the database, patients potentially eligible for the specific study will be contacted by the senior neurologist who will explain

the study and ascertains the patient's interest. If interested, the patients were examined in the clinical laboratories where more detailed evaluations were performed, so to confirm that fulfil the study criteria.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions The participants were randomly assigned in numerical order from 1 to 5. So, the one who was placed in number 1 started the exercise protocol first and the one in number 5 was the last one, with a difference of one week with each other.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Participants were allocated by generate random numbers using the ECXEL, after all participants assigned inform consent. Subsequently, all participants started from the baseline assessments. The intervention was

		introduced systematically in one patient, week by week, while baseline data collection continues in the others without any intervention.
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions All patients who fulfilled the inclusion criteria were randomized and then informed about the study procedures, so finally the consent for participation was given. Randomisation requested by the senior neurologist responsible for recruitment according inclusion/exclusion criteria from the Cyprus Institute of Neurology and Genetics. Then the list of participants' randomised order was sent to the main researcher (i.e., senior physiotherapist of the Cyprus Institute of Neurology and Genetics) who coordinated the study. The therapist gave the information about the exercise protocol and the related neurophysiological and clinical assessments.
Blinding (masking)	17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how Due to the nature of the study, which was a concurrent multiple baseline design across subjects, neither participants nor staff could be blinded to allocation. A certified fitness instructor designed the exercise protocols and two experienced physiotherapists performed all adequate assessments who fed all data into the computer separate datasheets so that the researchers could proceed with the analysis plan.

	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial Due to the nature of the study, which was a concurrent multiple baseline design across subjects, neither participants nor staff could be blinded.

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol **Primary Outcome Measures** Using electromyography (EMG) signals, we will analyse bilateral cortical excitability and bilateral central motor conduction time to determine corticospinal plasticity and therefore to test the primary hypothesis. **Secondary Outcome Measures** We investigated the effects of the specific exercises protocol on the resting motor threshold, on the motor evoke potential (MEP) amplitude and latency of Abductor Pollicis Brevis muscle, which defined cortical excitability. Clinical symptoms using clinical assessment (i.e., gait, balance, strength, hand dexterity, cognitive functions)

Mini Balance Evaluation Systems Test measures dynamic balance, functional mobility, and gait in neurological patients, including people with RRMS, the Six Spot Step Test is an assessment tool that evaluates a complex range of sensorimotor functions, part of which are lower limb strength, spasticity, coordination, as well as balance. We well assess the isometric muscle force of major muscle groups with the use of the muscle controller (Kinvent Biomechanique, Montpelier, France) which is a dynamometer used in the evaluation and rehabilitation of muscle strength that provides real time biofeedback. Also, we employed the oral form of the Symbol Digit Modalities Test which assesses the information processing speed. Finally, we performed the Modified Fatigue Impact Scale which is a short questionnaire that requires the participants to describe the effects of fatigue during the past four weeks.

Quality control

All scientific researchers are experienced and specialized in motor and cognitive functions in healthy individuals but also in patients with neurological and psychiatric disorders. Also, the two main organizations that work together to carry out this study are specialized in the field of rehabilitation of patients with chronic neurological diseases. The first institution is the Cyprus University of Technology and specifically the Department of Rehabilitation Sciences of the School of Health Sciences, has a well-equipped rehabilitation clinic in which systematic studies are carried out for the rehabilitation of neurological patients. The second institution, which is the Cyprus Institute of Neurology and Genetics, is one of the most recognized centers for providing health services in Cyprus to people with chronic neurological diseases. The staff of the

Neurophysiology laboratory and the Physiotherapy Unit of the Cyprus Institute of Neurology and Genetics, are specialized in evaluation and treatment services to people with chronic neurological diseases such as MS.

As depicted in the experimental procedures scheme (see Appendix 3_Figure 1), all patients began the baseline phase simultaneously and during this phase, each participant were assessed on primary and secondary outcome measures by two physiotherapists who are staff members of the Cyprus Institute of Neurology and Genetics. In order to ensure quality of the intervention a certified fitness instructor designed the protocols in collaboration with the senior physiotherapist of the Cyprus Institute of Neurology and Genetics. During the intervention phase we performed several neurophysiological (see primary outcome measures) and five clinical assessments (see secondary outcome measures) (i.e., once a week), to collect several data for every participant. Additionally, every participant underwent three follow-up assessments in total, after finishing the exercise protocol, to explore possible long-lasting effects. Each follow-up assessment included both primary and secondary outcome measures, performed by the two experienced physiotherapists.

Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols

18b

Due to our study design, several data collected from the beginning of the participants' allocation. Several clinical and neurophysiological assessment recorded for each participant. However, participants' had the opportunity to be systematically informed about their own clinical condition as well as the level of performance during the intervention phase. Another important factor which promoted participants' retention was the

		opportunity they had to exercise in a different way that they used to, as well as it was offered to them a scientific exercise program under supervision without any cost.
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol All information related to the specific program, including data for all participants, collected by the principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics, who participated in the research study. From the moment each participant was enrolled to the research study he / she received a participant ID number (e.g., 101, 102). From that point on, participant's name was not mentioned and the participant ID was used. Only the principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics has access to the name of the participants. Five years after the data collection, all the material stored electronically will be permanently deleted by the principal investigator. In addition, copies of the collected data (questionnaires, clinical and cognitive tests, forms, etc.) will be immediately destroyed. All data were stored in the office of the principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics. All computers that were used in this research study for data storage were offline. In addition, logging in to computers requires an account password that was known only to the two

		previously mentioned researchers of the study. Finally, the buildings are also protected by a security company during working hours.
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol To investigate possible effects of our protocol we followed recommended guidelines, in which we performed a separate analysis for each of the outcome measures, in all experimental phases (i.e., baseline, intervention and follow up). We performed a visual analysis first, in order to determine whether there was a functional relationship between the intervention and the outcome measures, and secondly, we performed a quantitative analysis method to evaluate the magnitude of the intervention effect, provided there was evidence from the visual analyses. We performed all neurophysiological and clinical assessments to each participant according to the number of data points during each phase (i.e., baseline, intervention, follow-up). Initially, a visual analysis was conducted and presented graphically in a spaghetti plot, in order to define whether there was a functional relation between the intervention and the outcome measures. During the visual analysis, six features of the research design graphed data were examined: level, trend, stability, immediacy of the effect, overlap, and consistency. Over the within-phase examination an evaluation of level, trend and stability were examined. Level was reported from the mean score of each dependent variable and trend determined whether the data points are monotonically decreased or increased. Stability was estimated based on

the percentage of data points falling within 15% of the phase median, if this is higher than 80% then we assume that this criterion was met. Additionally, over the between-phase examination an evaluation of overlapping data among baseline and intervention phases, consistency of data patterns and immediacy of effect were performed.

Secondly, in order to estimate the individual-level effect sizes, we used three different methods of quantitative analysis, as suggested by 'What Works Clearinghouse', the standardized mean difference (Cohen's d), the standardized mean difference with correction for small sample sizes (Hedges' g) and piecewise regression analysis which does not only reflect the immediate intervention effect, but also the intervention effect across time. Multilevel modelling, which is recommended by the 'What Works Clearinghouse' and the single case educational design, specific mean difference index was used to estimate the magnitude of the effect across cases and compared to the effect obtained by the single level estimates. All tests were two sided and statistical analysis were performed using the statistical software R (https://www.r-project.org/).

20b Methods for any additional analyses (e.g., subgroup and adjusted analyses)

N/A due to the study design.

	20c	Definition of analysis population relating to protocol non-adherence (e.g., as randomised analysis), and any statistical methods to handle missing data (e.g., multiple imputation) N/A due to the study design.
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed A data monitoring committee was not needed due to the study design, although a biostatistician, who was independent and blinded to the study procedures performed all statistical analysis. All data sheets which were completed throughout the assessments of the primary and secondary outcome measures were given to her in order to proceed with the adequate statistical analysis.

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial Due to the study design, an independent and blinded to the study procedures biostatistician performed the analysis of the data at the end of each phase (i.e., baseline, intervention, follow-up). The principle investigator had access to the results but without any possibility to terminate the trial until all participants complete the intervention phase.
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct An adverse event could be defined as any unpleasant medical occurrence in a subject. Adverse events could be collected after the subject had provided consent and enrolled in the study. If a subject experience an adverse event after the informed consent document was signed but the subject had not started to perform any intervention, the event could be reported as not related to the study. All adverse events occurring during the intervention could be recorded and reported to the senior neurologist. A serious adverse event for this study could be any untoward medical occurrence that was believed by the investigators to be causally related to study intervention and results in any of the following: Life-threatening condition (that is, immediate risk of death); severe or permanent disability, musculoskeletal pain, fatigue. Serious adverse events occurring after a subject is discontinued from the study would NOT be reported unless the investigators feel that the event may had been

		caused by the exercise protocol or any of the study procedures. Investigators would determine relatedness of an event to study intervention, as well as whether the event was unexpected or unexplained given the subject's clinical course and previous clinical conditions. Due to the study design, participants were clinically examined systematically, thus there was a systematic monitoring so any possible adverse event could be reported.
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor The principal investigator and the steering committee, who were members of the entire research team, were frequently (i.e., once a week) audit the overall quality and completeness of the data, examined source documents and confirmed that all health professionals who were included in the study had complied with the requirements of the protocol. Also, they reviewed all source documents as needed, whether data sheets were completed and were updated.
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval This protocol and the template informed consent forms contained in Appendix 3 were reviewed and approved by the Cyprus National Bioethics Committee with respect to scientific content and compliance with applicable research and human subjects' regulations. The protocol, site-specific informed consent forms (local language

		and English versions), participant education and recruitment materials, and other requested documents also were reviewed and approved by the ethical review bodies. The principal investigator made safety and progress reports to the Cyprus National Bioethics Committee at least annually and within three months of study termination or completion.
Protocol amendments	25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, patient population, sample sizes, study procedures, or significant administrative aspects could be approved by the Cyprus National Bioethics Committee prior to implementation.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) The principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics introduced the study procedures to the participants. Participants also received information sheets and then were able to provide written consent from since are adults without any cognitive impairment. All information sheets and consent form are written in local language which is Greek).

	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial Participants' study information will not be released outside of the study without the written permission of the participant. Only the principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics will have access to the name of the participants. Five years after the data collection, all the material stored electronically will be permanently deleted by the principal investigator. In addition, copies of the collected data (questionnaires, clinical and cognitive tests, forms, etc.) will be immediately destroyed. All data stored in the office of the principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics. All computers which were used in this research study for data storage were offline. In addition, logging in to computers requires an account password that was known only to the two previously mentioned researchers of the study. Finally, the buildings are also protected by a security company during working hours.

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site Principal investigator declared no conflict of interest.
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators The steering group had access to the full trial dataset in order to ensure the overall results. To ensure confidentiality, data dispersed to project team members were blinded of any identifying participant information.
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Patients who were enrolled into the study are covered by through the standard General Health System.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions The scientific integrity of the project requires that all the data from all participants were analysed and reported to all team members after completion of the experimental procedures.

Each paper or abstract were reviewed or/and approved by the steering committee, before being submitted to an appropriate scientific journal or/and a scientific conference.

The entire research study terminated at the planned target of 1,5 years after the last participant will be enrolled to the intervention phase. Regardless of the timing and circumstances of the end of the study, close-out proceeded in two stages:

- The first stage was the interim period for analysis and documentation of study results.

- The second stage was the debriefing of participants and dissemination of study results, in which the paper with the final results was submitted to an appropriate journal. We expected to take about 5 to 6 months, after the last participant finished the follow up phase, to compile the final paper.

31b Authorship eligibility guidelines and any intended use of professional writers

Principal investigator, steering and data manager committee were the lead authors of the entire research study. In case of some protocol authors were not named authors of subsequent publications, their role in protocol design was acknowledged in the published report.

Appendices

Informed consent materials

Biological specimens

- Study type:	
Estimated Enrollment:	Interventional 5 participants
Allocation:	N/A
Intervention Model:	Single Group Assignment
Intervention Model Description:	The study follows a concurrent multiple baseline design across subjects, which involves five people with RRMS that will be managed as five different case studies. The specific design has the advantage to verify the cause-effect inference clearly by the staggered duration through separate baseline phases
Masking:	None (Open Label)
Masking Description:	Participants are people with multiple sclerosis according inclusion/exclusion criteria. Investigators are health professionals (i.e., physiotherapist, sports scientist, neuropsychologist, neurologist, biostastician).

Primary Purpose:	Basic Science
- Date of first enrolment:	N/A
- Sample size:	5
- Recruitment status of this trial:	Participants were recruited and enrolled
- Primary Outcome(s):	Central Motor Conduction Time [Time Frame: Through study completion, an average 35 weeks]
- Key secondary outcomes:	Resting Motor Threshold, Motor Evoked Potential Amplitude and Latency, Mini Balance Evaluation Systems Test, Six Spot Step Test, Action Research Arm Test, Hand Held Dynamometer, Symbol Digit Modalities Test, Modified Fatigue Impact Scale [Time Frame for all measures: Through study completion, an average 35 weeks].

- Ethics Review:	Status: Registration No. ΕΕΒΚ ΕΠ 2022 32 Date of approval: 10/6/2022 Name and contact details: Cyprus National Bioethics Committee; 22 Laertou Str., 2365 Ayios Dometios, Nicosia, (00357) 22-809038 / 22-809039 / 22819101 cnbc@bioethics.gov.cy
- Completion date:	July 2021 is the estimated study completion date.
- IPD sharing statement:	Undecided

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Appendix 2a: Informed Consent Form

CONSENT FORMS **for participation in a research program** (The forms are comprised of 10 pages)
Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

This form provides the explanations in plain and comprehensible language regarding what is being requested from you and/or what will happen to you if you agree to join the program:

1. All risks that may exist or any inconvenience you may incur from participating in the program.
2. The person(s) who will have access to your information and will arise from the program you will take part in and/or other material/data that you voluntarily provide for the program.
3. The time period during which the Principal Investigator will have access to your information and/or material concerning you.
4. What the Principal Investigator hope to learn as a result of your participation.
5. Estimation of the benefit that can be gained for researchers and/or sponsors of this program.
6. **You should not participate if you do not wish to, or if you have any concerns about your participation in the program.**
7. If you decide to join, you must indicate if you have participated in any other research programs within the last 12 months.
8. If you decide not to participate and you are a patient, your treatment will not be affected by your decision.
9. **You are free to withdraw your consent to participating in the programme at any time.**
10. If you are a patient, your decision to withdraw your consent will not have any effect on your treatment.
11. All pages of consent forms must bear your full name and signature.

Principal Investigator of the Program you are invited to participate in
Dr. Nikos Konstantinou, Assistant Professor, Department of Rehabilitation Sciences, Cyprus University of Technology. Vragadinou 15, Limassol, 3041, telephone number: 00357 25002294, email: nikos.konstantinou@cut.ac.cy

Surname:	.....	Name:	....
Signature:	.....	Date:	....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.
Program Duration:
5 years

Do you give consent for yourself or for someone else?	
If you have responded for another person, please provide details and name.	

Question	YES or NO
Did you fill in your consent forms personally?	
Over the past 12 months, have you been involved in any other research program?	
Did you read and understand the information regarding patients and/or volunteers?	
Have you had the opportunity to ask questions and discuss the Program?	
Have you been given satisfactory answers and explanations to any of your questions?	
Do you understand that you can withdraw from the programme whenever you wish?	
Do you understand that if you withdraw, you do not need to give any explanations for your decision?	
(For patients) do you understand that, if you withdraw, there will be no impact on any treatment you get or you can get in the future?	
Do you agree to join the program?	

With whom did you speak with?			
Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate

Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

Brief description of the program (procedures and purpose).

You are invited to participate in a research of the Department of Rehabilitation Sciences of the Cyprus University of Technology (CUT) in collaboration with the Cyprus Institute of Neurology and Genetics (CING). Before deciding whether or not to participate, it is important to understand the main goal of this research study. Take some time to carefully read the information below, as well as you can discuss it with others if you wish. Moreover, you can ask our team if there is anything that is not clear or you do not understand or if you would like more information about this information sheet or consent form. Take time to decide whether or not you want to participate.

The main goal of this research study, is to investigate the effects on the clinical condition and quality of life in patients with Multiple Sclerosis (MS), throughout a program of different types of exercises (i.e., in-phase bilateral exercises), which are adapted to different sports activities and fitness exercises. The study is expected to be an important tool in the implementation of future treatment programs in patients with MS. Participation in this research is voluntary. You are not expected to receive any immediate financial or personal benefit; however, your participation will greatly contribute to the development of science.

Risks of participation: There are no risks and no complications from your participation in this study.

Confidentiality: The data collected is anonymous and you do not need to provide any information about your identity. No information received will be able to identify you.

Research participation procedure: The duration of this exercise program is 12 weeks for each participant who will take part in three weekly sessions (45-60 minutes / session). Furthermore, before the beginning of the intervention, during the 12 weeks of the intervention and in one year after the end of the intervention, there will be frequent clinical assessments of motor and cognitive functions, neurophysiological examinations (corticospinal plasticity), as well as recording of results from questionnaires concerning the quality of life and fatigue for each participant.

Right of non-participation or withdrawal: Your participation is completely voluntary and you should participate only if you wish. Choosing not to participate or leave during the research program will not have a negative effect on you, will not cost you anything and will not affect any other treatment you may receive.

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

Details of what will be requested and/or what will happen to program participants
If you agree to participate in this research study, we will ask you to come to CING. This research study includes frequent assessments before, during and after the end of a 12-week program based on sports and functional training exercises. More specifically, in the Physiotherapy Unit of ING, frequent clinical assessments and the sessions of the specific exercises will be performed based on protocol. Also, in the Neurophysiology lab of CING will be performed the neurophysiological assessments with the use of Transcranial Magnetic Stimulation (TMS). During each clinical assessment you will be asked to perform specific and very reliable motor tests, such as balance, strength, gait and functionality of the upper limbs. You will also be asked to complete a cognitive skills test for the evaluation of the information processing speed. It is important to mention that between each test you will be given enough time to rest so as to avoid fatigue. Specialist physiotherapists will record your results in each test and at the end of the program if you wish you can be given all the findings. Thru each neurophysiological assessment, measurements will be recorded in relation to the corticospinal plasticity of the two cerebral hemispheres and the central motor conduction time of the upper limbs via TMS. At this point we will ask you to sit comfortably in an armchair in order to activate the motor cortex of the brain. Using special electrodes that will be placed in both hands, we will monitor the activity of a muscle in each hand respectively. When applying TMS, we will activate your brain cells with simple magnetic pulses produced by an insulated coil which we will place on your scalp. Each pulse travels through your scalp, causing a small electric current in the cortex (the outer part of the brain). The goal is to find the area of the brain that corresponds to the specific muscle of the hand in which we have placed the special electrode. It is important to know that magnetic pulses can cause a slight tingling

sensation on your scalp. This sensation is usually not unpleasant but sometimes it can actually cause an annoying sensation.

The exercise program in which you will be invited to take part, includes exercises that contain in-phase bilateral movements and are adapted to different sports activities and functional exercises. All exercises will be under the guidance and supervision of specialized sport scientist and physiotherapists experienced in the field of neurorehabilitation and you will need to wear the appropriate sportswear.

The purpose of this research study is to investigate the effect of specific exercises on your clinical condition as well as on the functioning of the central nervous system. It is important to know that you can request to stop and leave this program at any time without any excuse and with no consequences.

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

Details of any risks that may exist or any inconvenience that program participants may incur
By providing a series of exercises for a long time, you may feel tired. In case this happens, you will be given specific guidelines in order to recover. All researchers are health professionals and experienced in the field of neurorehabilitation and exercising in chronic diseases.

Details of what information and/or what material will be collected under the program, who will have access to it and for how long.
You will not be asked for any personal data that could lead to your identification. During the program, all data will be collected in relation to your motor and cognitive condition, as well as your motor cortex activity via TMS. At the same time, you will be given the Safety Check Form for TMS, which you will be asked to state if you have a history of specific diseases.

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

WHERE APPLICABLE, FUTURE STORAGE AND USE OF BIOLOGICAL SAMPLES AND PERSONAL DATA: Please note and sign either left or right	
Except for the purposes of this program that will last for ... . years **I consent**: ☐ Signature:	Except for the purposes of this study that will last for ... . years **I do not consent**: ☐ Signature:
that my biological samples (buccal swabs, saliva or DNA) and genetic information which shall be stored at the …… **may be kept for more than …… years and be used in future studies** upon authorization of the Cyprus National Bioethics Committee (CNBC), following the relevant application for renewal by the Principal Investigator of this Program, I understand that matters of confidentiality will always be in force.	

If new information that directly affects your health is discovered, would you like to be informed?		
YES ☐	NO ☐	I CANNOT MAKE A DECISION NOW. PLEASE ASK AGAIN IF NEEDED ☐

Details of what data will be generated for you within the program, who will have access to them and for how long.
All data that will be gained, are related to motor and cognitive skills, as well as to the motor cortex activity. Only the research team will have access to this data and it will be destroyed after 5 years. In case of publication of the results of the present study in a scientific journal or in any other conference, any of your personal data will not be published and you will not be able to identify yourself with any published material.

Surname:	……	Name:	……
Signature:	……	Date:	……

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.
Expected benefit for participants
The benefits that you will have with your participation in this research study is the systematic evaluation of your clinical condition for a year, as well as your participation for a long time in an exercise program that will keep you physically active with possible improvement of your motor and cognitive functions and improving your quality of life.
Expected benefit for researchers and/or sponsors
All results from the study, will contribute to the scientific knowledge but also to the practical application by health professionals, regarding the effect of this type of movements and exercises in order to improve the current clinical situation of people with MS. Moreover, they will offer the advantage for better quality of rehabilitation in possible future progression of the disease.
Details of termination or early postponement of the research program.
All data that will collected from your assessments will be stored in the laboratory of the rehabilitation clinic, at the School of Health Sciences, of the CUT, as well as in the office of the senior physiotherapist of the Physiotherapy Unit, of CING. All the data that will be received within the specific research program will be destroyed after 5 years.
Description of procedures of handling data and/or biological samples of participants who withdraw from the study prior to its completion.
The data of the participants who will withdraw from the study before its completion will be destroyed immediately and will not be used in any study process. Specifically, any data stored on the researchers' computers will be deleted when you leave the study by the PI and any printed material collected will be destroyed and recycled.

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

Full contact details and title of the person to whom participants can submit complaints or grievances regarding the program they participate in.
Dr. Charalambos Charalambous Head of the department of research and international collaborations Cyprus University of Technology Telephone number: 00357 25 002562 email: c.chrisostomou@cut.ac.cy

Full contact details and title of the person whom participants can contact for more information or clarifications about the research program.
Dr. Nikos Konstantinou Assistant Professor, Department of Rehabilitation Sciences, Cyprus University of Technology. Vragadinou 15, Limassol, 3041 telephone number: 00357 25002294 email: nikos.konstantinou@cut.ac.cy

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

Appendix 2b: Information sheet about TMS

- TMS is a non-invasive method of diagnosis and /or treatment of the motor cortex of the brain.

- The clinical use of TMS for diagnostic purposes is the ability to measure brain activity and connect specific areas of the nervous system.

- Throughout TMS it is possible to detect basic pathophysiological changes that cause disorders due to dysfunction of the motor cortex of the brain, resulting from certain diseases or injuries.

- In summary, the use of TMS for diagnostic purpose, is the investigation of the connection between the primary motor cortex (which is responsible for muscle movements) and the muscles, in order to assess the damage caused by stroke, diagnosis of multiple sclerosis and amyotrophic lateral sclerosis associated with neuronal death disorders, motor disorders, and general brain injuries.

- TMS affects the electrical activity of the brain through a pulsed magnetic field. The magnetic field is generated by transmitting current pulses through an insulated coil which has a circular or butterfly shape.

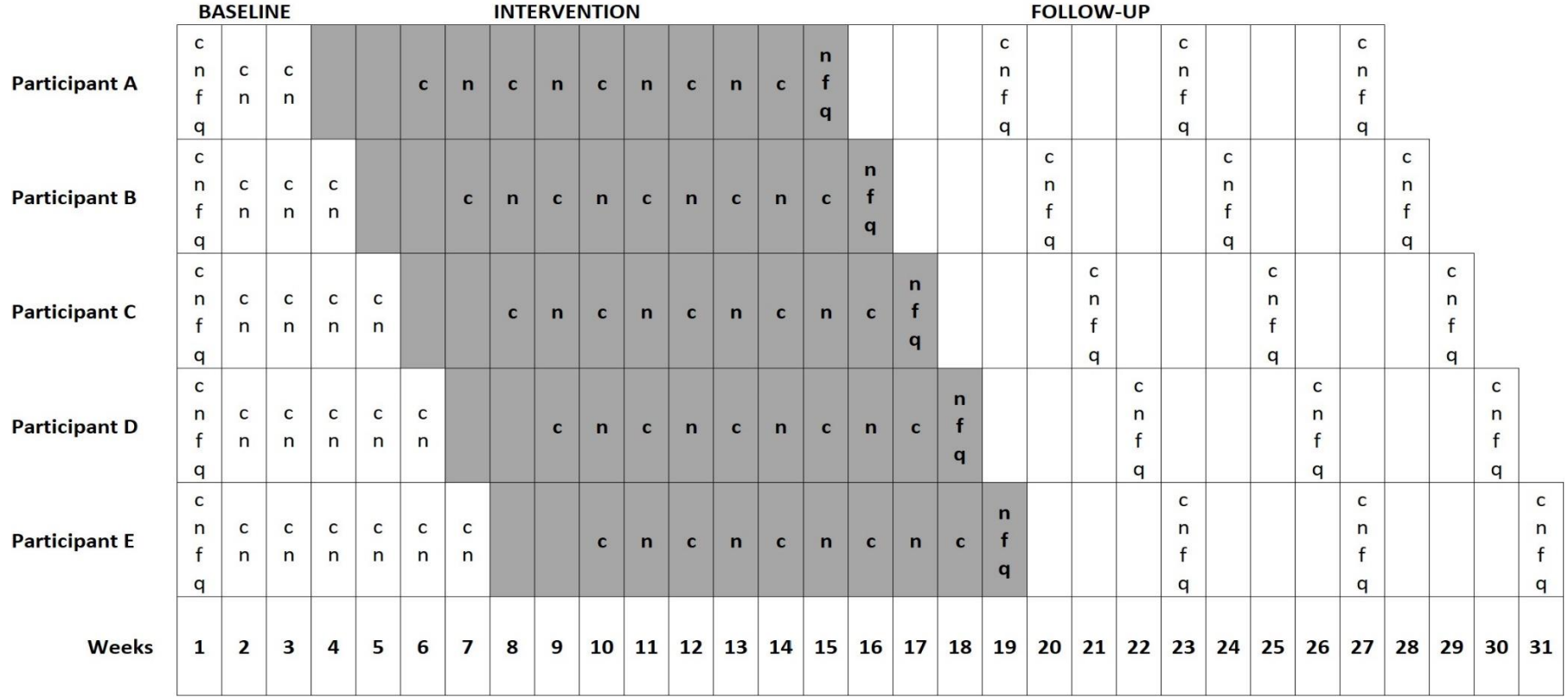
- The coil is wrapped in plastic and placed close to the scalp so that the magnetic field can focus on specific areas of the cerebral cortex.

- The magnetic field created in TMS, can penetrate the scalp without pain and safely to activate specific neurons (brain cells).

- The use of TMS has been extensively validated in clinical trials and medical research studies, and has been approved for use in various countries (e.g., Canada since 2002 and later in the USA).

- Several scientific articles refer to the encouraging results of TMS in the treatment of Parkinson's disease, Multiple Sclerosis, Schizophrenia with Acoustic hallucinations, Rehabilitation after stroke and other emotional disorders.

Appendix 3: Figure 1

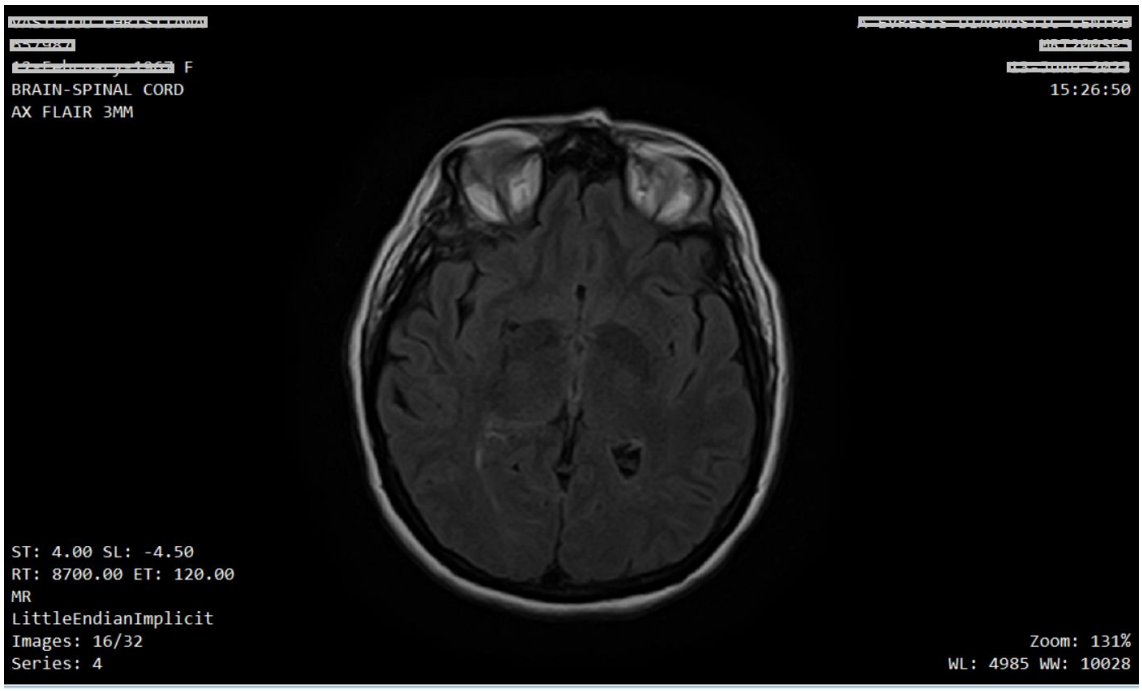


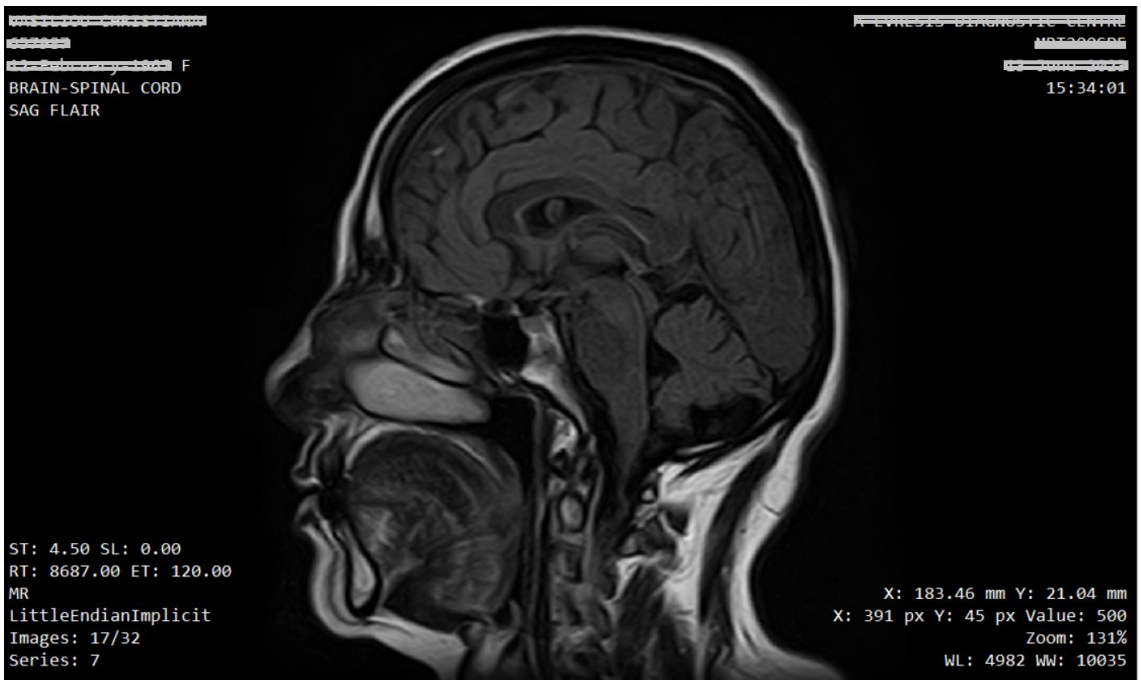
Note: c; clinical assessment, n; neurophysiological assessment via TMS f; Modified Fatigue Impact Scale questionnaire, q; Medical Outcomes Study Questionnaire Short Form 36 Health Survey. Grey color represents the intervention phase. Each row (A – E) represents a different participant. Every cell represents a different week, so every procedure which is included (i.e., c, n, f, q) was performed during the corresponding week but in different days.

APPENDIX VII

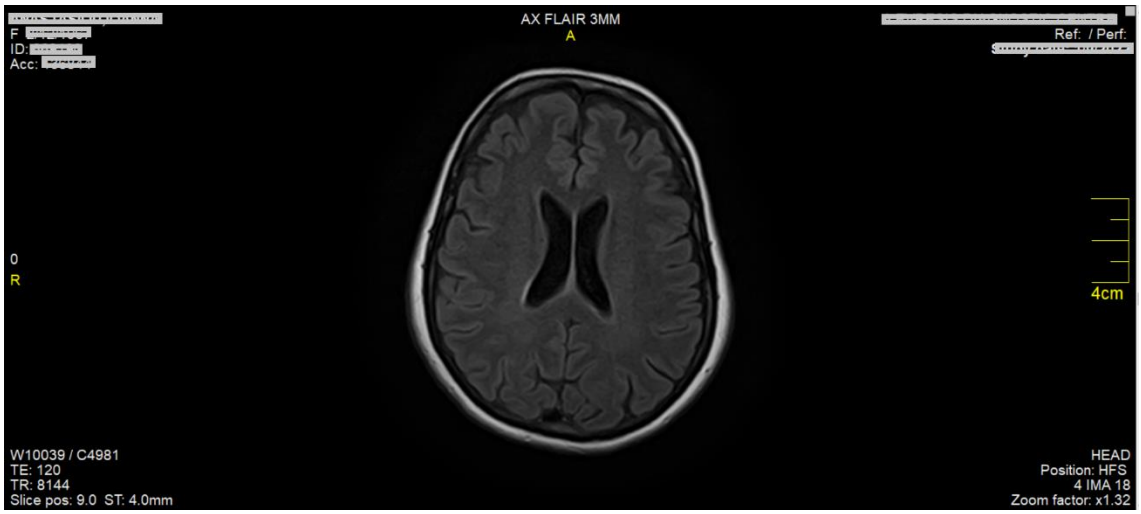
Individual MRI slices

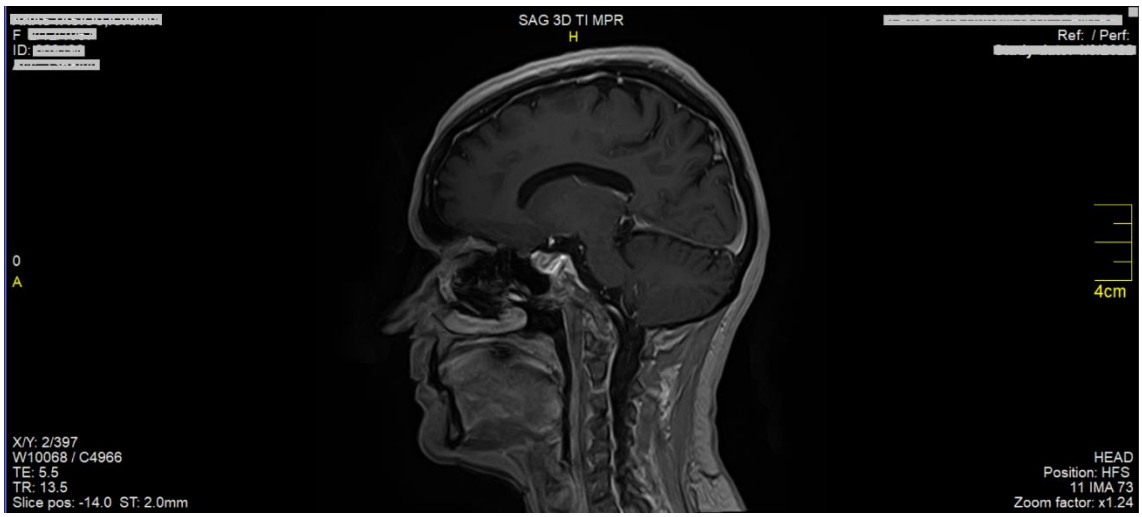
Participant A



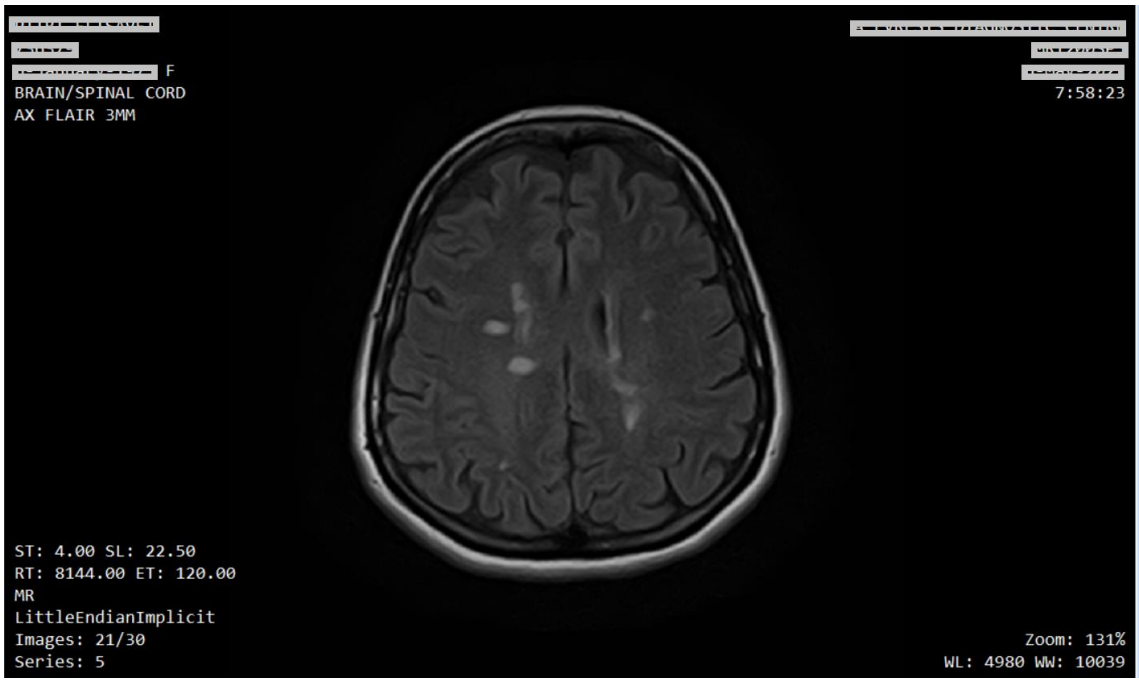


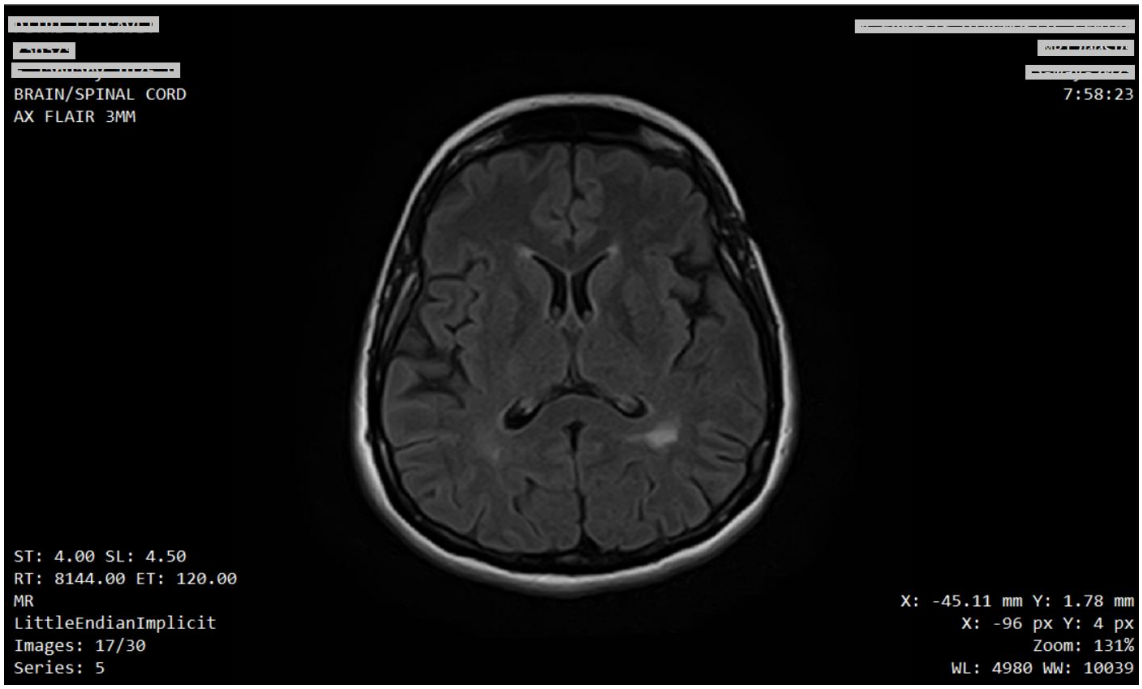
Participant B



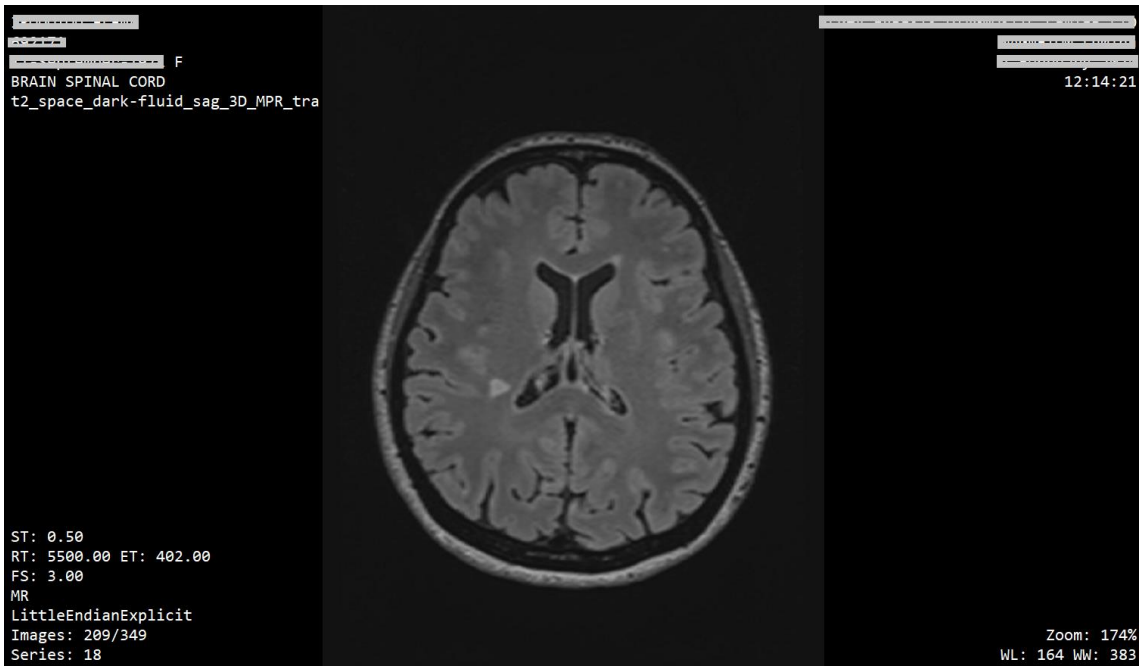


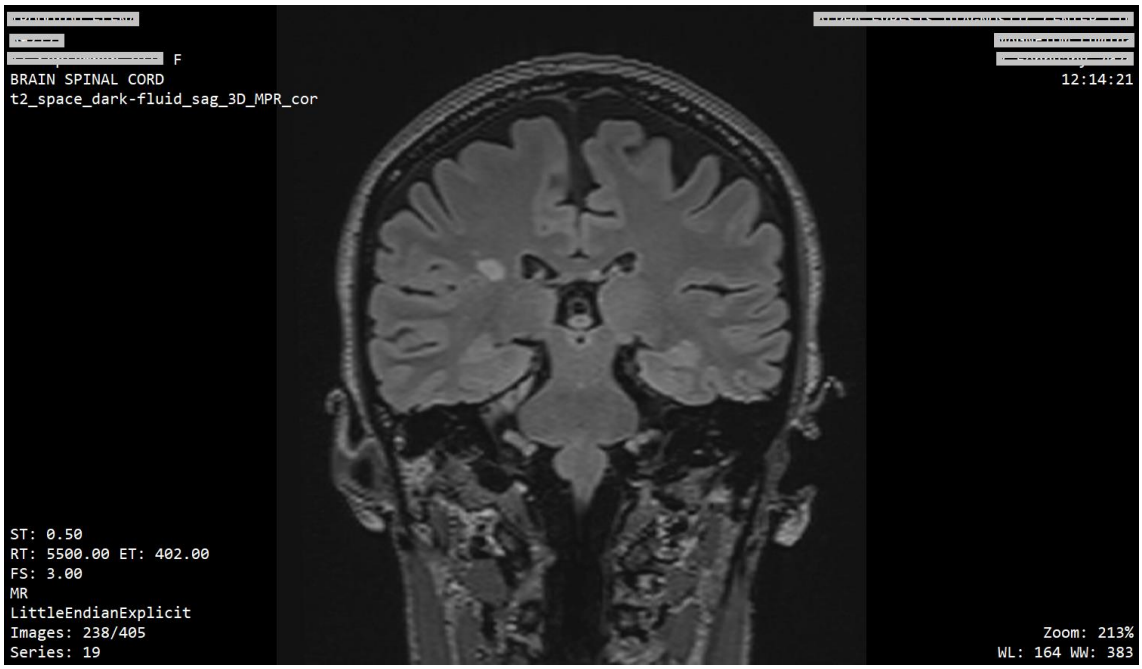
Participant C



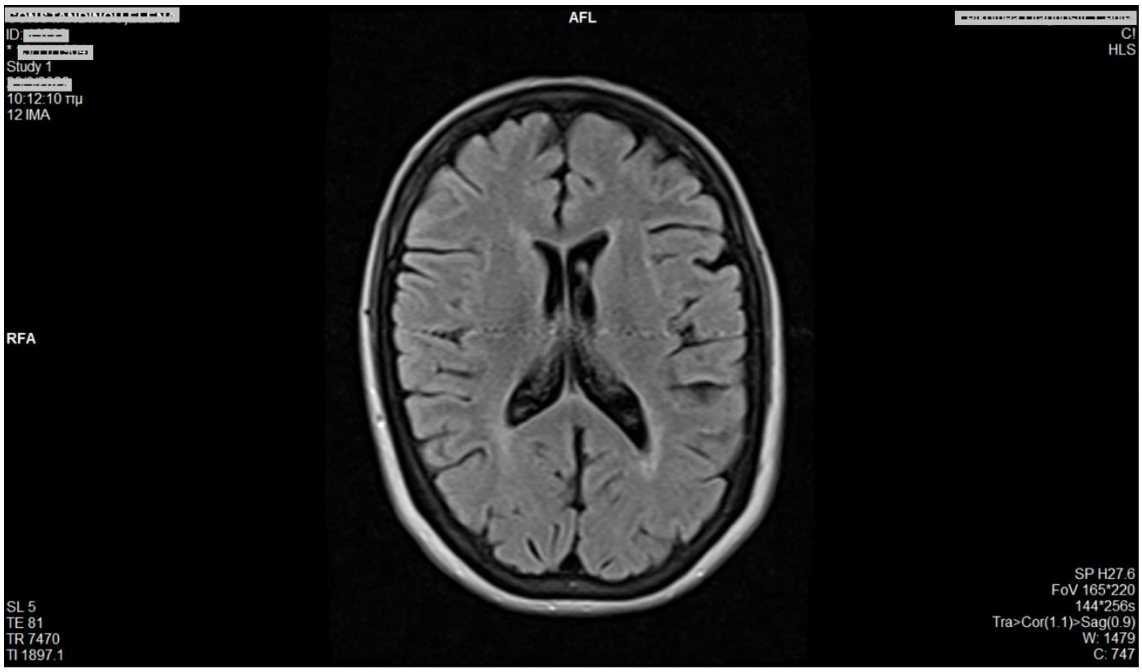


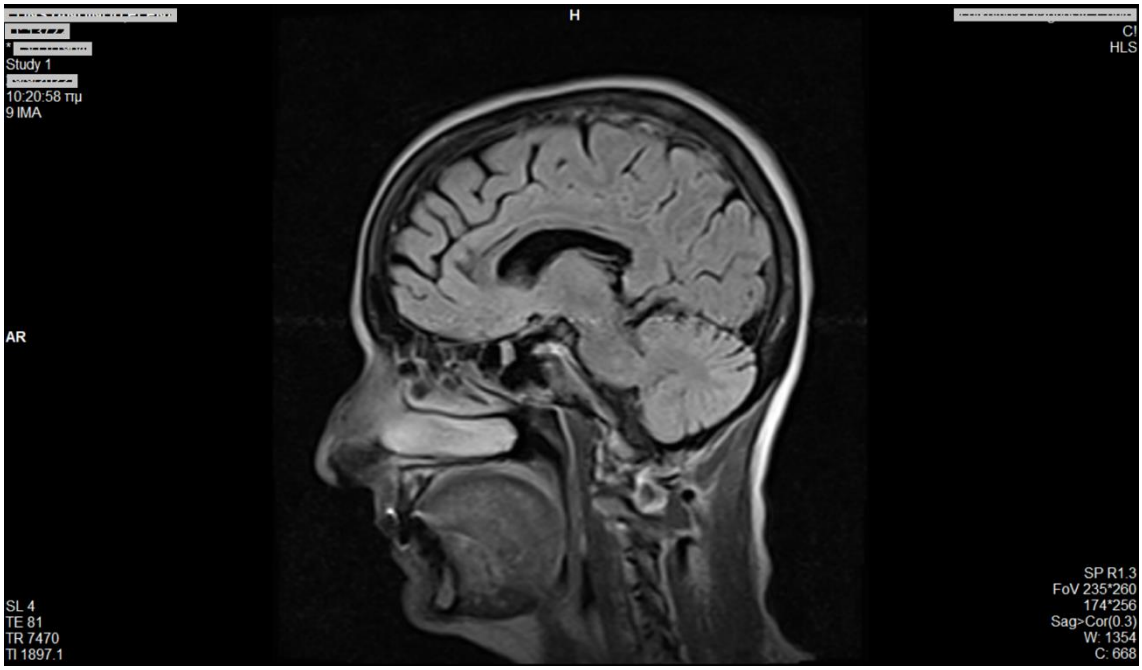
Participant D





Participant E





**Note:* All MRIs were not performed by the same diagnostic center

APPENDIX VIII

Multiple Baseline Design – Results.

Table 1. Individual Central Motor Conduction Time data across all assessment points during baseline, intervention and follow-up phases – multiple baseline study.

Participant	A	B	C	D	E
Left APB					
Assessment point	**Baseline phase**				
1	8.7	9.9	9.7	18.7	7.8
2	8.8	8.4	11.4	17.7	10.8
3	10.9	8.5	11.4	14.8	7.4
4	N/A	8.7	21.3	8.9	11.1
5	N/A	N/A	11	17	8.1
6	N/A	N/A	N/A	7.9	5.7
7	N/A	N/A	N/A	N/A	*
Mean	9.5	8.9	13	14.2	8.5
Assessment point	**Intervention phase**				
1	14.4	7.1	17.1	15.5	7.2
2	8.5	10.9	9.4	17.9	11.4
3	7.9	17	10.1	5	8.5
4	9.6	17.2	8.6	11.3	1**
5	9.3	10.8	9.9	8.4	11.6
Mean	9.9	12.6	11	11.6	9.7
Assessment point	**Follow-up phase**				
1	9.8	8.9	8	10.3	10.5
2	10.9	16.7	11.1	16.5	12.1

3	10.1	11	11.8	12.6	*
Mean	10.3	12.2	10.3	13.1	11.3
Right APB					
Assessment point	**Baseline phase**				
1	8.5	8.7	10.6	9	8.8
2	7.8	7.5	14.7	8.6	13
3	7	19.1	11.4	13.2	3.3**
4	N/A	10.4	11.2	15.5	8.3
5	N/A	N/A	12.5	8.3	8.5
6	N/A	N/A	N/A	17.3	8.1
7	N/A	N/A	N/A	N/A	*
Mean	7.7	11.4	12.1	12	9.3
Assessment point	**Intervention phase**				
1	4**	8	12.2	14.3	5.9
2	7.9	8.8	12	17.1	6.5
3	5.3	9.3	12.1	14.6	7.2
4	10.2	8.4	10.4	12	6.9
5	5.9	8	12.8	9.3	9
Mean	7.3	8.5	11.9	13.4	7.1
Assessment point	**Follow-up phase**				
1	8.6	8.5	11.8	19.2	7.5
2	8.7	8.7	11.9	23.8	11.1
3	9.1	8.2	11.2	10.6	*
Mean	8.8	8.5	11.6	17.9	9.3

Table 1: APB; Abductor Pollicis Brevis, N/A; Non-Applicable, Ex; Exclusion. Individual data of CMCT (ms) for each phase are presented in Table 1. The assessment points during baseline phase were recorded according to the study design (i.e., concurrent multiple baseline design across subjects). (*) refers to the assessment points which were missed. Participant E couldn't perform the last TMS assessment of baseline and follow-up phases, on both upper limbs. (**) refers to the data points (i.e., left APB, fourth intervention point of participant E; right APB, first intervention point of participant A; right APB, third baseline point of participant E) which were excluded from the analysis, because they didn't satisfy our criteria during data collection. Mean values of each phase were used for the analysis of the CMCT. Prior to averaging, we manually rejected TMS-elicited waveforms with MEPs peak to peak amplitudes less than 50mV and MEPs latencies higher or lower than two standard deviations; therefore, we calculated the mean of the values derived from the remaining trials. By doing this, we ensure data quality and reliability.

Table 2. Individual Motor Evoked Potentials Amplitude data across all assessment points during baseline, intervention and follow-up – multiple baseline study.

Participant	**A**	**B**	**C**	**D**	**E**
Left APB					
Assessment point	**Baseline phase**				
1	0.9	0.05	0.05	0.07	0.2
2	0.2	0.1	0.2	0.02	0.2
3	0.5	0.05	0.2	0.05	0.3
4	N/A	0.1	0.07	0.06	0.1
5	N/A	N/A	0.1	0.08	0.2
6	N/A	N/A	N/A	0.2	0.2
7	N/A	N/A	N/A	N/A	*
Mean	0.5	0.08	0.1	0.08	0.2
Assessment point	**Intervention phase**				
1	0.9	0.05	0.05	0.06	0.1
2	0.1	0.1	0.2	0.06	0.1

3	0.06	0.06	0.08	0.1	0.09
4	0.2	0.07	0.1	0.2	0.05
5	0.01	0.06	0.07	0.3	0.08
Mean	0.1	0.07	0.1	0.1	0.09
Assessment point	**Follow-up phase**				
1	0.3	0.05	0.09	0.06	0.1
2	0.3	0.05	0.1	0.05	0.08
3	0.07	0.08	0.1	0.08	*
Mean	0.2	0.06	0.1	0.06	0.09
Right APB					
Assessment point	**Baseline phase**				
1	0.8	0.06	0.5	0.09	0.2
2	0.3	0.1	0.08	0.08	0.07
3	0.2	0.1	0.2	0.05	0.06
4	N/A	0.05	0.2	0.06	0.2
5	N/A	N/A	0.1	0.2	0.2
6	N/A	N/A	N/A	0.1	0.2
7	N/A	N/A	N/A	N/A	*
Mean	0.47	0.09	0.2	0.1	0.1
Assessment point	**Intervention phase**				
1	0.06	0.08	0.1	0.08	0.1
2	0.06	0.1	0.1	0.05	0.2
3	0.06	0.06	0.1	0.07	0.1
4	0.3	0.3	0.1	0.05	0.1
5	0.2	0.09	0.1	0.07	0.2

Mean	0.06	0.1	0.1	0.07	0.1
Assessment point	**Follow-up phase**				
1	0.2	0.1	0.2	0.05	0.2
2	0.6	0.09	0.3	0.05	0.06
3	0.1	0.1	0.07	0.09	*
Mean	0.3	0.1	0.2	0.06	0.1

Table 2: APB; Abductor Pollicis Brevis, N/A; Non-Applicable. Individual data of MEPs amplitude (mV) for each phase are presented in Table 2. The assessment points during baseline phase were collected according to the study design (i.e., concurrent multiple baseline design across subjects). (*) refers to the assessment points which were missed. Participant E couldn't perform the last TMS assessment of baseline and follow-up phases, on both upper limbs. Mean values of each phase were used for the analysis of the MEPs amplitude. Prior to averaging, we manually rejected TMS-elicited waveforms with MEPs peak to peak amplitudes less than 50mV and MEPs latencies higher or lower than two standard deviations; therefore, we calculated the mean of the values derived from the remaining trials. By doing this, we ensure data quality and reliability. Throughout the within and between-subjects' visual analysis, data variability and instability could be observed to all participants, during all study phases and for both upper limbs.

Table 3. Individual Motor Evoked Potentials Latency data across all assessment points during baseline, intervention and follow-up phases – multiple baseline study.

Participant	**A**	**B**	**C**	**D**	**E**
Left APB					
Assessment point	**Baseline phase**				
1	24.2	22.8	23.2	34.3	23.5
2	25	21.3	24.3	32.2	23.4
3	26.2	21.3	24.9	31.3	23.6
4	N/A	21.7	35.4	25.5	26

5	N/A	N/A	24.2	30.4	23.4
6	N/A	N/A	N/A	23.9	21.4
7	N/A	N/A	N/A	N/A	*
Mean	25.1	21.8	26.4	29.6	23.5
Assessment point	**Intervention phase**				
1	30.7	20.1	30.3	30.2	22.4
2	24.5	23.8	22.3	34.4	25.5
3	23	29.8	23.6	18.6	23
4	24.6	28.2	23.8	26.2	15.5
5	25.1	23.5	22.8	23.5	26.5
Mean	25.6	25.1	24.5	26.6	22.6
Assessment point	**Follow-up phase**				
1	24.9	22.4	21.3	25.5	22.9
2	24.7	28.1	23.2	28.5	25.5
3	24.4	24.7	25.4	25.9	*
Mean	24.6	25	23.3	26.6	24.2
Right APB					
Assessment point	**Baseline phase**				
1	24.3	21.9	24.5	24.7	22.5

2	23.6	20.5	28.1	23.9	27
3	22.8	32.2	24.5	28.8	18.8
4	N/A	23.7	24.3	29	22.9
5	N/A	N/A	24.2	21.5	23.7
6	N/A	N/A	N/A	31.9	22.3
7	N/A	N/A	N/A	N/A	*
Mean	23.6	24.6	25.1	26.6	22.9
Assessment point	**Intervention phase**				
1	18.8	21.1	25.1	29.5	20.5
2	23.8	21.8	24.7	30.4	21.3
3	20.7	22.5	25.4	29.2	21.5
4	25.7	21.5	23.4	26.7	21.7
5	19.8	21.6	25.9	24.1	23.3
Mean	21.7	21.7	24.9	28	21.6
Assessment point	**Follow-up phase**				
1	23.8	21.9	24.6	32.4	22
2	24.2	20.8	24.6	37.9	25
3	24.8	22.1	24.6	23.8	*
Mean	24.3	21.6	24.6	31.3	23.5

Table 3: APB; Abductor Pollicis Brevis, N/A; Non-Applicable. Individual data of MEPs latency (ms) for each phase are presented in Table 3. The assessment points during baseline phase were collected according to the study design (i.e., concurrent multiple baseline design across subjects). (*) refers to the assessment points which were missed. Participant E couldn't perform the last TMS assessment of baseline and follow-up phases, on both upper limbs. Mean values of each phase were used for the analysis of the MEPs latency. Prior to averaging, we manually rejected TMS-elicited waveforms with MEPs peak to peak amplitudes less than 50mV and MEPs latencies higher or lower than two standard deviations; therefore, we calculated the mean of the values derived from the remaining trials. By doing this, we ensure data quality and reliability. Throughout the within and between-subjects' visual analysis, data variability and instability could be observed to all participants, during all study phases and for both upper limbs.

Table 4. Individual Resting Motor Threshold data across all assessment points during baseline, intervention and follow-up phases.

Participant	**A**	**B**	**C**	**D**	**E**
Left APB					
Assessment point	**Baseline phase**				
1	63	67	74	42	69
2	60	68	73	48	78
3	63	69	71	42	65
4	N/A	70	72	43	76
5	N/A	N/A	75	45	63
6	N/A	N/A	N/A	49	69
7	N/A	N/A	N/A	N/A	*
Mean	**62**	**68.5**	**73**	**44.8**	**70**
Assessment point	**Intervention phase**				

1	48	54	62	41	59
2	43	56	44	40	56
3	40	61	56	37	54
4	45	57	59	60	31
5	46	58	54	47	55
Mean	**44.4**	**57.2**	**55**	**45**	**51**
Assessment point	**Follow-up phase**				
1	57	65	64	78	64
2	61	70	56	69	64
3	57	63	65	75	*
Mean	**58.3**	**66**	**61.6**	**74**	**64**
Right APB					
Assessment point	**Baseline phase**				
1	58	65	66	59	78
2	56	61	63	63	68
3	53	62	67	57	70
4	N/A	65	61	63	64
5	N/A	N/A	65	59	64
6	N/A	N/A	N/A	67	71

7	N/A	N/A	N/A	N/A	*
Mean	**55.6**	**63.2**	**64.4**	**61.3**	**69.1**
Assessment point	**Intervention phase**				
1	25	59	60	62	58
2	41	57	56	53	58
3	23	60	58	50	59
4	42	58	57	60	47
5	21	57	56	48	60
Mean	**30.4**	**58.2**	**57.4**	**54.6**	**56.4**
Assessment point	**Follow-up phase**				
1	56	61	52	78	64
2	51	67	57	71	65
3	57	65	55	70	*
Mean	**54.6**	**64.3**	**54.6**	**73**	**64.5**

Table 4: APB; Abductor Pollicis Brevis, N/A; Non-Applicable, Individual data of MEPs latency (ms) for each phase are presented in Table 4. The assessment points during baseline phase were collected according to the study design (i.e., concurrent multiple baseline design across subjects). (*) denotes the missed assessment points, which Participant E couldn't perform (i.e., last TMS assessment of baseline and follow-up phases bilateral). Throughout the within and between-subjects' visual analysis, data variability and instability could be observed to all participants, during all study phases and for both upper limbs.

Table 5. Mini Balance Evaluation Test, Six Spot Step Test and Symbol Digit Modalities Test, individual clinical measures data across all assessment points during baseline, intervention and follow-up phases – multiple baseline study.

Participant	**A**	**B**	**C**	**D**	**E**
Mini Balance Evaluation Test (maximum score = 28)					
Baseline phase	22	23	19	21.4	20
Intervention phase	27	24.8	21.4	24.6	25
Follow-up phase	26.3	23.3	18.3	21.6	22.3
NAP / standard error	1 / 0.06	1 /0.04	1 /0.03	1 /0.02	1 /0.02
p-value <	0.05	0.01	0.01	0.01	0.01
Six Spot Step Test					
Baseline phase	7	10.4	11	7.8	9.5
Intervention phase	5.6	8.5	9.8	6.4	7.5
Follow-up phase	5.6	8.6	10.4	6.6	7.6
NAP / standard error	1 / 0.06	1 /0.04	1 /0.03	1 /0.02	1 /0.02
p-value <	0.05	0.01	0.01	0.01	0.01
Symbol Digit Modalities Test					
Baseline phase	73.6	69.5	71.2	51.7	54.5
Intervention phase	79.8	73.6	79.8	65.6	63.2
Follow-up phase	76	71	72	60.6	56.3
NAP / standard error	1 / 0.06	1 /0.04	1 /0.03	1 /0.02	1 /0.02
p-value <	0.05	0.01	0.01	0.01	0.01

Table 5: NAP; Non-Overlap of All Pairs. Mean scores and statistical analysis of the clinical measures are presented in Table 5. In the statistical analysis, the values of NAP and p-value refer to the results from the comparison between baseline and intervention phase only. The follow-up phase was not included in the statistical analysis. The Mini Balance Evaluation Test assesses the dynamic balance, functional mobility and gait, which showed a significant improvement to all participants during the intervention phase. However, during the follow-up phase there was a reduction in the performance of all participants and return to the baseline values as were expected, since there was no exercise during that phase. The Six Spot Step Test which examines the gait, showed significant improvement to all participants during the intervention phase and lasted across the follow-up phase as well. The Symbol Digit Modalities Test which assesses the information processing speed, showed significant improvement to all participants during the intervention phase, with a tendency to return to the baseline level during the follow-up phase.

Table 6. Individual Action Research Arm Test data across all assessment points during baseline, intervention and follow-up phases – multiple baseline study.

Participant	**A**	**B**	**C**	**D**	**E**
Left Upper Limb (maximum score = 57)					
Baseline phase	8.7	9.6	9.8	11.4	13.4
Intervention phase	6.6	8	8.8	8	8.7
Follow-up phase	6.7	8.1	9.7	9.6	10.6
NAP /standard error	1/ 0.06	1/0.04	1/0.03	1/0.02	1/0.02
p-value <	0.05	0.01	0.01	0.01	0.01
Right Upper Limb (maximum score = 57)					
Baseline phase	8	9	10.1	10.3	12.2
Intervention phase	5.8	7.9	8.4	8.7	8.4
Follow-up phase	6.6	8	8.8	9.5	9.2
NAP /standard error	1/ 0.06	1/0.04	1/0.03	1/0.02	1/0.02
p-value <	0.05	0.01	0.01	0.01	0.01

Table 6: NAP; Non-Overlap of All Pairs. Mean scores and statistical analysis of the Action Research Arm Test, for both upper limbs and for each participant, are presented in Table 6. In the

statistical analysis the values of NAP and p-value refer to the results from the comparison between baseline and interventions phase only. A significant improvement was observed to all participants, in both upper limbs, during the intervention phase. However, during the follow-up phase there was a reduction for both left and right upper limbs in the performance (i.e., increased values) of all participants, which return to the baseline values as were expected, since there was no exercise.

Table 7. Individual data of Muscle Strength Test across all assessment points during baseline, intervention and follow-up phases – multiple baseline study.

Participant	**A**		**B**		**C**		**D**		**E**	
	L	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**
Shoulder Flexors										
Baseline phase	10.1	12.3	10.3	11.9	10.9	12	12.2	13.3	10.7	12.1
Intervention phase	15.8	19.3	16	19.4	13.1	14.5	14.4	17.6	14.8	15.4
Follow-up phase	13.4	16.2	14.2	17.6	12	12.7	12.3	15.7	13.5	12.9
NAP /standard error	1/0.06	1/0.06	1/0.04	1/0.04	1/0.03	1/0.03	1/0.02	1/0.02	1/0.02	1/0.02
p-value <	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Shoulder Extensors										
Baseline phase	15.3	15.1	15	16.1	12.6	11.9	15.6	15.6	13.1	14.8
Intervention phase	20.8	21.3	20.9	21.6	14.5	15.2	19.2	20	17	17.3
Follow-up phase	18.1	16.7	19.1	20	13.3	13.9	18.1	18.1	15.7	16.5

NAP /standard error	1/0.06	1/0.06	1/0.04	1/0.04	1/0.03	1/0.03	1/0.02	1/0.02	1/0.02	1/0.02
p-value <	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Shoulder Internal Rotators										
Baseline phase	8.6	13.4	10.3	11.4	8.9	10.1	11.4	14.4	8	12.5
Intervention phase	13.2	16.8	13.9	16.8	12.6	12.7	14.7	18.2	12.9	18.2
Follow-up phase	10.8	15.2	12.8	14.6	11.5	12.1	13.1	17.2	11.4	16.9
NAP /standard error	1/0.06	1/0.06	1/0.04	1/0.04	1/0.03	1/0.03	1/0.02	1/0.02	1/0.02	1/0.02
p-value <	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Shoulder External Rotators										
Baseline phase	9.1	9.2	11.4	11.3	10	10.5	11.5	12.3	9.9	11.5
Intervention phase	12	12.2	12.7	13.1	12.4	13.2	15.6	14.7	12.5	13.7
Follow-up phase	11.4	11.9	11.3	11.6	11.4	12	14.8	13.1	11.5	12
NAP /standard error	1/0.06	1/0.06	1/0.04	1/0.04	1/0.03	1/0.03	1/0.02	1/0.02	1/0.02	1/0.02
p-value <	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Shoulder Adductors										

Baseline phase	13.5	13.2	12.6	13.7	10.3	12.1	17.1	17.2	12.2	12.3
Intervention phase	17.9	20.1	18.2	20.1	12.6	14.4	21.4	21.4	19.7	21.5
Follow-up phase	17.2	18.7	16.3	18.9	11	13	19.4	19.5	17.8	16.8
NAP /standard error	1/0.06	1/0.06	1/0.04	1/0.04	1/0.03	1/0.03	1/0.02	1/0.02	1/0.02	1/0.02
p-value <	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Shoulder Abductors										
Baseline phase	10	12.6	11.2	13.9	10.1	11.3	11.1	13.2	12.3	13.7
Intervention phase	16.7	18.8	17	19	13.2	12.8	17	17.8	17.3	16.8
Follow-up phase	15.1	15	16.1	17.8	12.3	11	15.8	16.2	14.5	14.3
NAP /standard error	1/0.06	1/0.06	1/0.04	1/0.04	1/0.03	1/0.03	1/0.02	1/0.02	1/0.02	1/0.02
p-value <	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Shoulder Horizontal Adductors										
Baseline phase	9.5	9.1	10.4	10.3	9.6	9.3	11.4	12	9.2	11.5
Intervention phase	12.7	13.3	13	13.4	11.1	12.7	14	13.8	12.5	15.2
Follow-up phase	11	9.8	11.2	12.4	10.8	11.3	12.3	12.5	10.9	13.3

NAP /standard error	1/0.06	1/0.06	1/0.04	1/0.04	1/0.03	1/0.03	1/0.02	1/0.02	1/0.02	1/0.02
p-value <	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Shoulder Horizontal Abductors										
Baseline phase	13.6	14	12.2	12.4	9.6	10.3	13.4	14.2	9.4	12.2
Intervention phase	17.3	19.4	17.6	19.6	11.3	13.3	16.1	19.9	12.4	14.6
Follow-up phase	15.5	17.2	16.4	18.2	10.4	11.8	14.5	19.1	10.5	12.9
NAP /standard error	1/0.06	1/0.06	1/0.04	1/0.04	1/0.03	1/0.03	1/0.02	1/0.02	1/0.02	1/0.02
p-value <	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Elbow flexors										
Baseline phase	15.2	14.5	12	16.7	12	12.5	12.3	14.2	12.1	15.2
Intervention phase	21.6	23	22.1	23	16.6	18.3	17.2	17.3	18.4	22.3
Follow-up phase	18.9	15.5	20.8	21.6	15.6	16.7	14.8	14.4	16.3	18.6
NAP /standard error	1/0.06	1/0.06	1/0.04	1/0.04	1/0.03	1/0.03	1/0.02	1/0.02	1/0.02	1/0.02
p-value <	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Elbow extensors										

Baseline phase	11	12.2	10.5	12.3	9.4	9.8	11.5	12.8	10.2	12
Intervention phase	17.7	21.8	17.3	21.5	11.7	13.5	15.4	17.4	17.5	17.2
Follow-up phase	11.9	13.3	15.3	19.1	10.2	12.2	14.3	16.1	15	15.3
NAP /standard error	1/0.06	1/0.06	1/0.04	1/0.04	1/0.03	1/0.03	1/0.02	1/0.02	1/0.02	1/0.02
p-value <	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Hand Grip										
Baseline phase	18.4	20.7	10.3	10.3	12.8	16.4	20.9	25.5	15.5	18.1
Intervention phase	23.5	25.1	23.6	25.4	15.3	18.6	25.4	28.3	20.5	25.4
Follow-up phase	20.8	23.8	22.4	23.4	14.1	17.7	23.8	25.9	16.5	23.1
NAP /standard error	1/0.06	1/0.06	1/0.04	1/0.04	1/0.03	1/0.03	1/0.03	1/0.02	1/0.02	1/0.02
p-value <	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Table 7: L; Left Upper Limb, R; Right Upper Limb, NAP; Non-Overlap of All Pairs, Mean scores (expressed in kg) and statistical analysis of the dynamometer test for each participant. All participants showed significant improvement for both left and right upper limbs, during the intervention phase. During the follow-up phase there was a reduction for both left and right upper limbs in the performance of all participants, which return to the baseline values as we were expected, since there was no exercise. In the statistical analysis the values of NAP and p-value refer to the results from the comparison between the data of the baseline and the intervention phase only.

Table 8. Individual Modified Fatigue Impact Scale data across all assessment points during baseline, intervention, and follow-up phases – multiple baseline study.

Participant	A	B	C	D	E
Baseline phase	43	53	45	45	44
Intervention phase	15	41	20	20	30
Follow-up phase_1	18	46	29	29	31
Follow-up phase_2	18	50	32	32	34
Follow-up phase_3	19	52	34	34	35

Table 8: Scores of the Modified Fatigue Impact Scale. All patients reported an improvement (i.e., decrease of values) regarding their fatigue level. However, after the intervention phase all participants, except the participant B, reported that their fatigue level still was improved. Participant B returned (i.e., increase of values) to its baseline level.

Table 9. Individual data of the Medical Outcomes Study Questionnaire Short Form 36 Health Survey across all assessment points during baseline, intervention, and follow-up phases – multiple baseline study.

Participant	A	B	C	D	E
Baseline phase	100	85	95	85	85
Intervention phase	104	92	99	92	93
Follow-up phase_1	101	89	97	92	91
Follow-up phase_2	100	86	95	89	91
Follow-up phase_3	101	87	94	87	92

Table 9: Scores of the Medical Outcomes Study Questionnaire Short Form 36 Health Survey. All patients reported an improvement (i.e., increase of values) regarding their QoL level. After the intervention phase all participants reported that their QoL still was improved.

APPENDIX IX

Individual EMG raw data

All participants ware coded as IBMS_*participant's letter* (e.g., IBMS_A,B,C,D and E)

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Patient Data

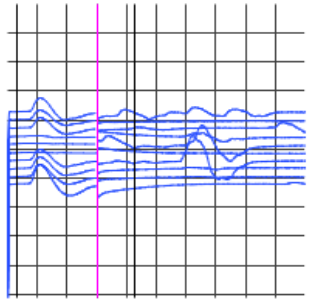
Patient ID: IBMS_A_Baseline#1
Name:
DOB:
Age:
Sex: Female
Height:

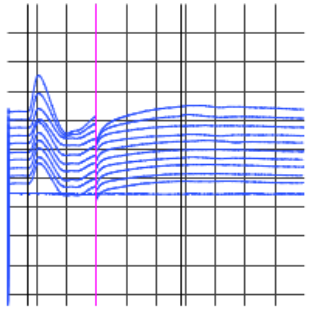
Date of Study: 10/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							21.4		
Median F-Response Right									
Wrist - APB							29.2		

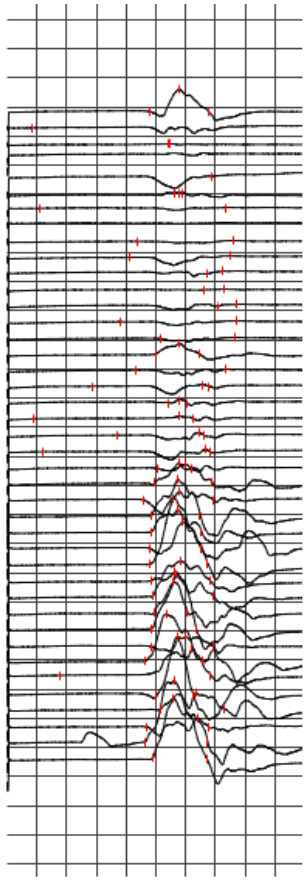
F-Wave

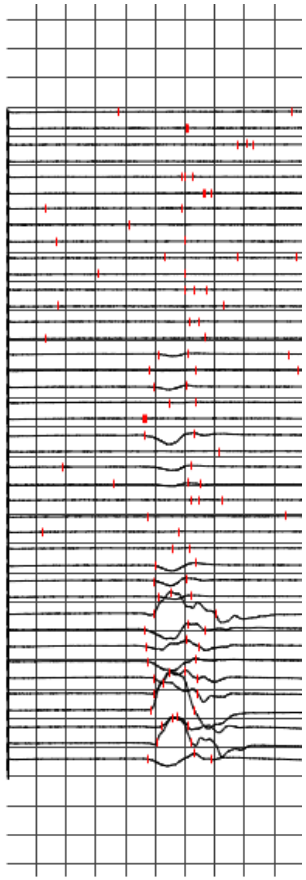




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	24.4	1.03	
		Right	23.7	1.47	
		Left-Right	0.70	0.44	





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Patient Data

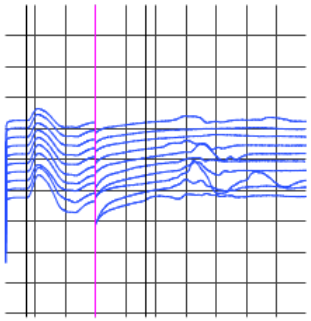
Patient ID: IBMS_A_Baseline#2
Name:
DOB:
Age:
Sex: Female
Height:

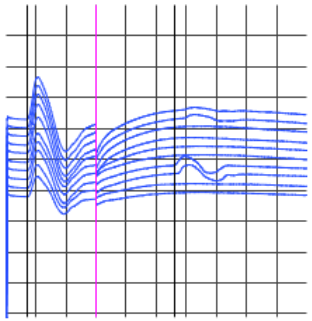
Date of Study: 17/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							23.4		
Median F-Response Right									
Wrist - APB							28.1		

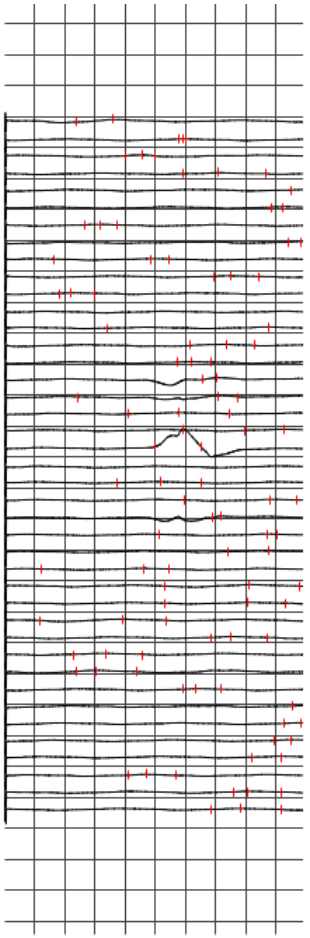
F-Wave

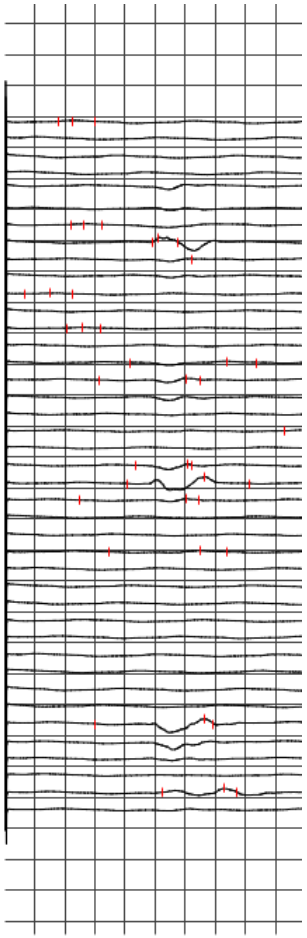




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	27.3	0.22	
		Right	--	--	
		Left-Right	27.3	0.22	





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Patient Data

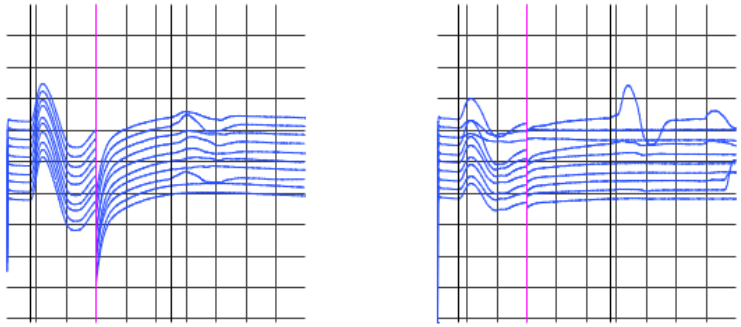
Patient ID: IBMS_A_Baseline#3
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 22/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

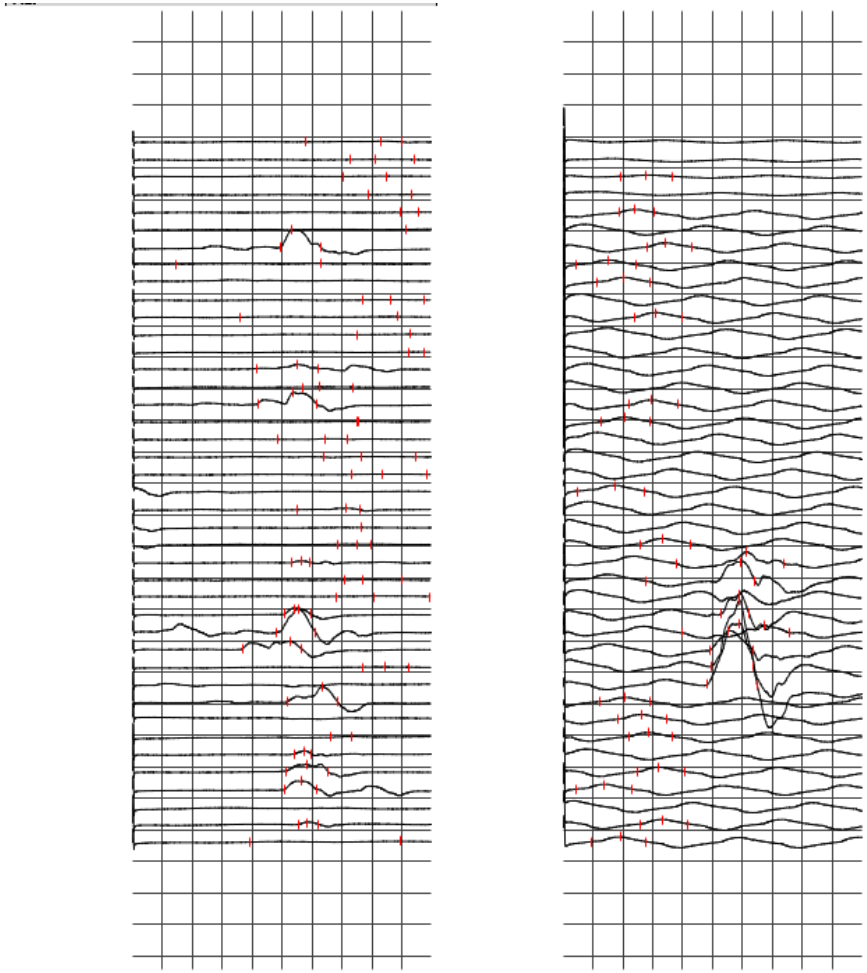
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							27.5		
Median F-Response Right									
Wrist - APB							28.9		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	27.6	0.038	
		Right	--	--	
		Left-Right	27.6	0.038	



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Patient Data

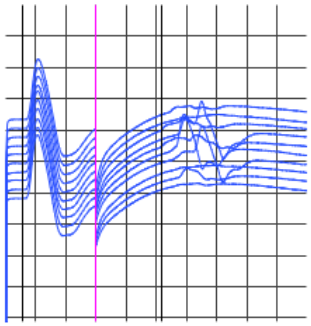
Patient ID: IBMS_A_Intervention#1
Name:
DOB:
Age:
Sex: Female
Height:

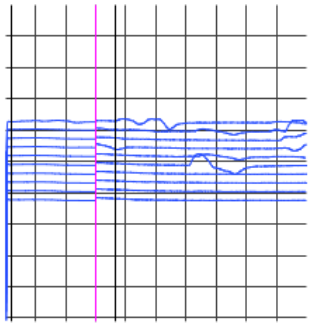
Date of Study: 19/04/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							25.9		
Median F-Response Right									
Wrist - APB							18.4		

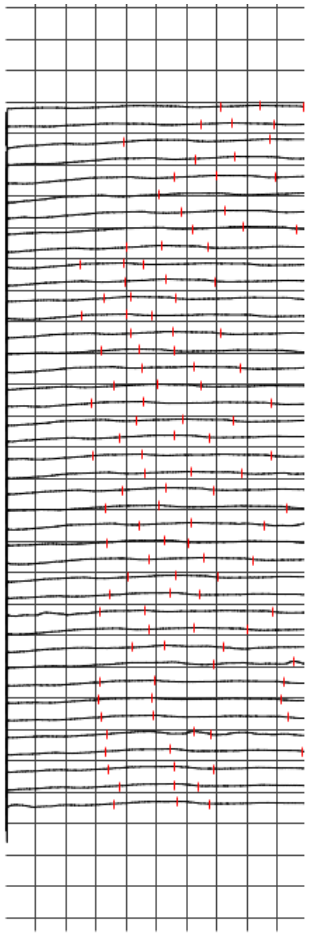
F-Wave

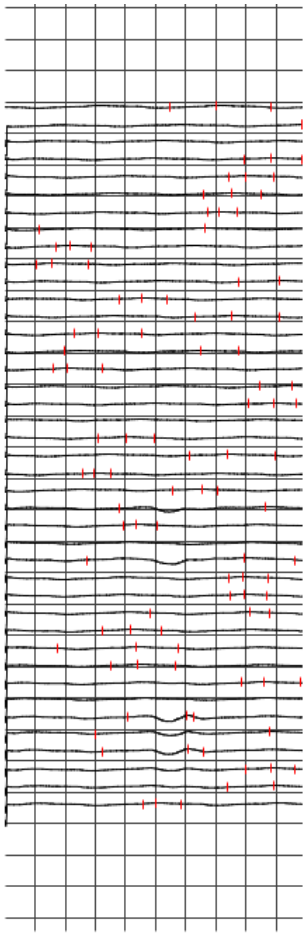




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	49.0	0.073	
		Right	--	--	
		Left-Right	49.0	0.073	





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Patient Data

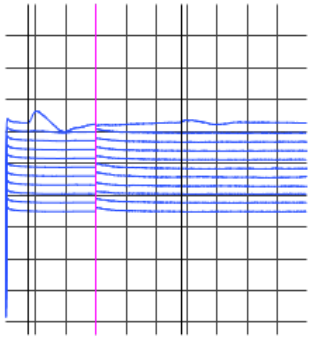
Patient ID: IBMS_A_Intervention#2
Name:
DOB:
Age:
Sex: Female
Height:

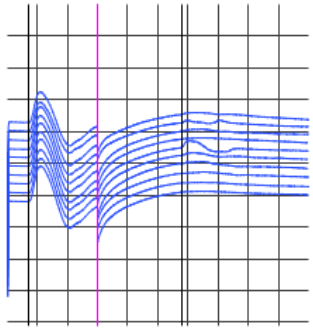
Date of Study: 05/05/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							29.1		
Median F-Response Right									
Wrist - APB							29.0		

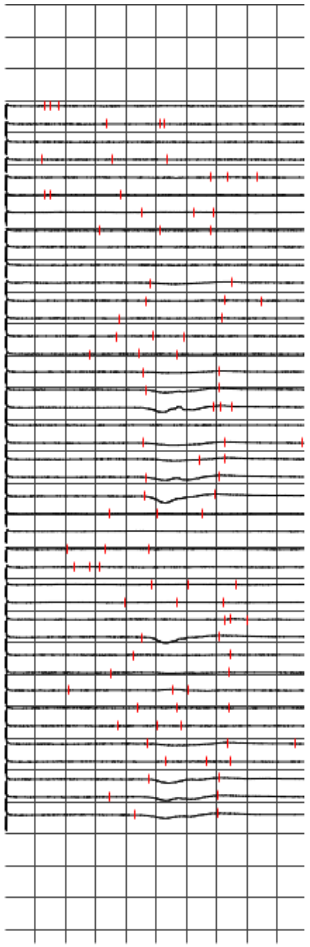
F-Wave

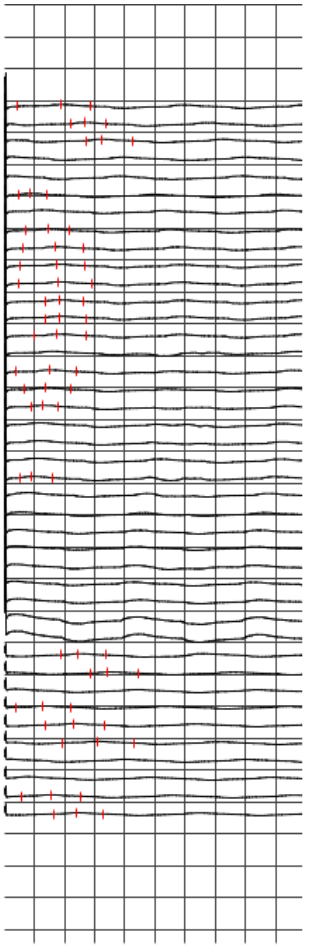




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	23.8	0.031	
		Right	4.6	0.071	





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Patient Data

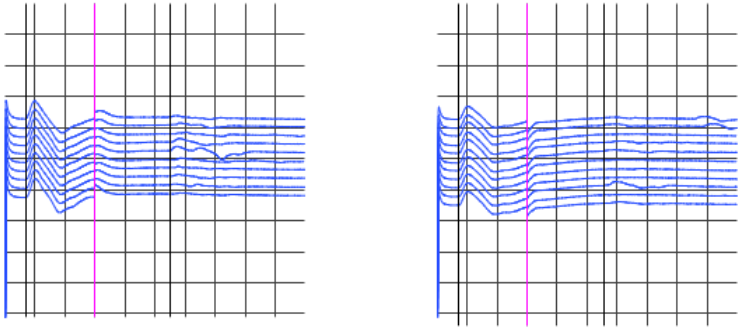
Patient ID: IBMS_A_Intervention#3
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 17/05/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

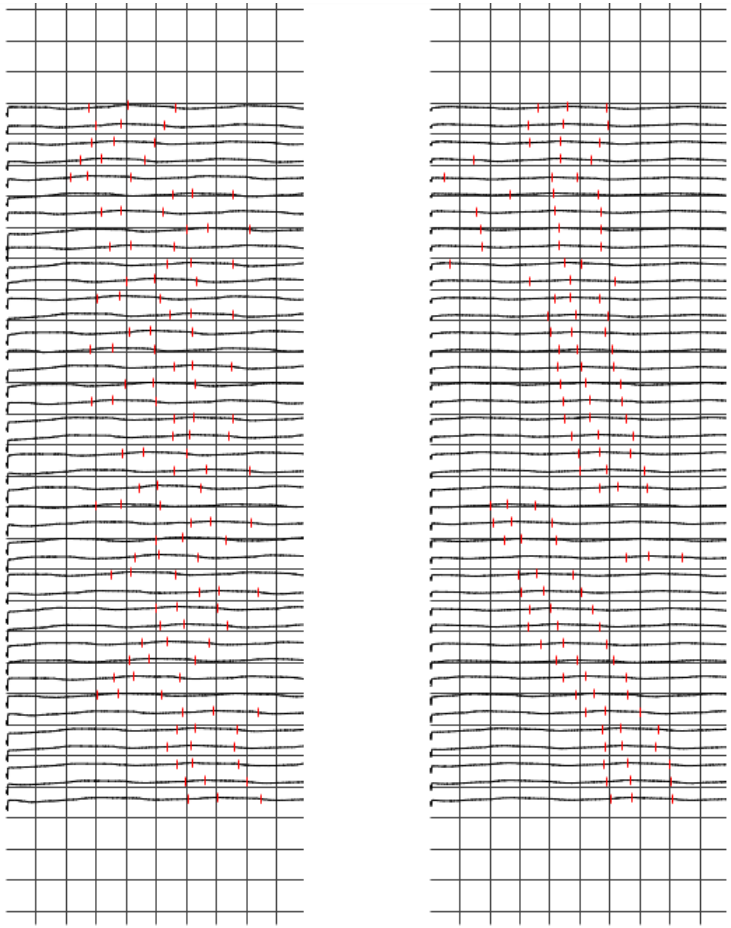
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							27.5		
Median F-Response Right									
Wrist - APB							27.7		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	20.1	0.050	
		Right	19.1	0.050	
		Left-Right	1.00	0	



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Patient Data

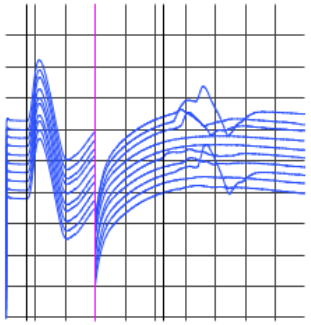
Patient ID: IBMS_A_Intervention#4
Name:
DOB:
Age:
Sex: Female
Height:

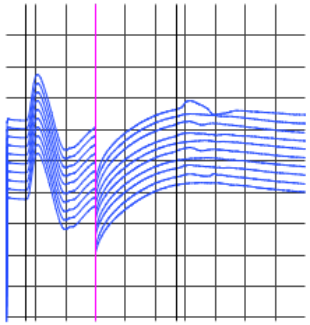
Date of Study: 31/05/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							26.3		
Median F-Response Right									
Wrist - APB							28.5		

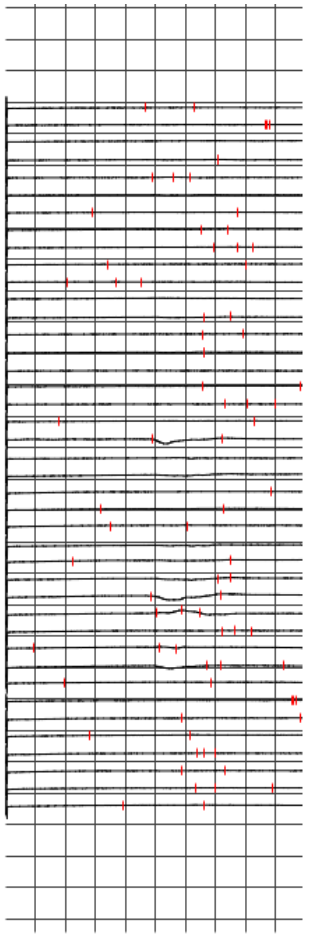
F-Wave

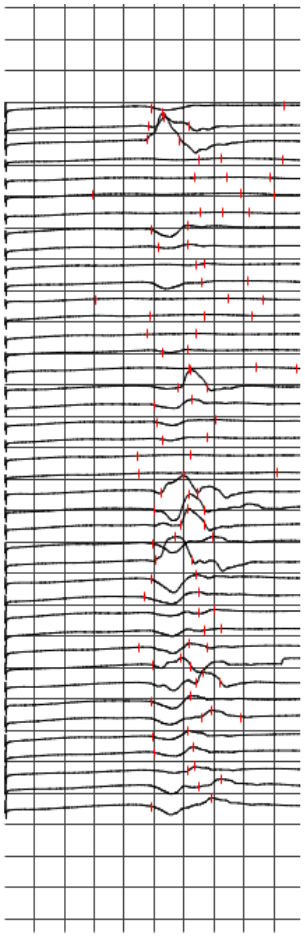




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	OCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	24.6	0.17	
		Left-Right	24.6	0.17	





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Patient Data

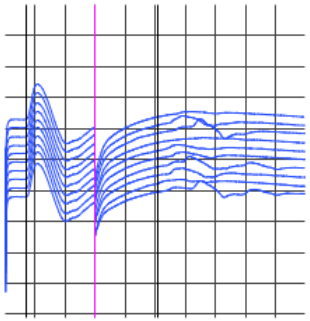
Patient ID: IBMS_A_Intervention#5
Name:
DOB:
Age:
Sex: Female
Height:

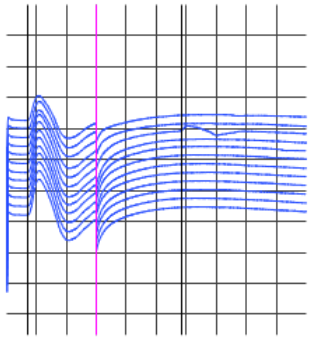
Date of Study: 14/06/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							25.4		
Median F-Response Right									
Wrist - APB							29.1		

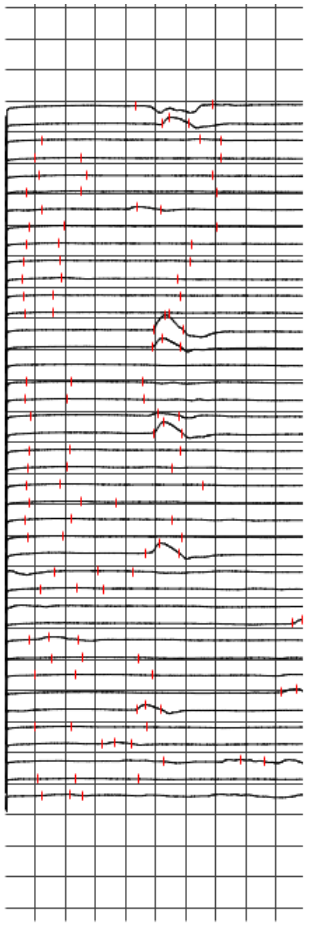
F-Wave

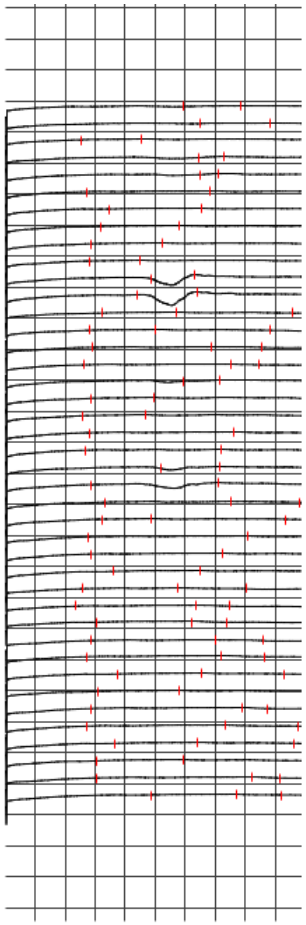




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	25.2	0	
		Left-Right	25.2	0	





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Patient Data

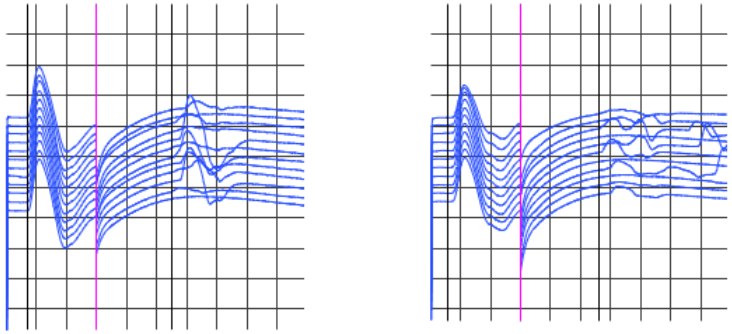
Patient ID: IBMS_A_Follow-up#1
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 14/07/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

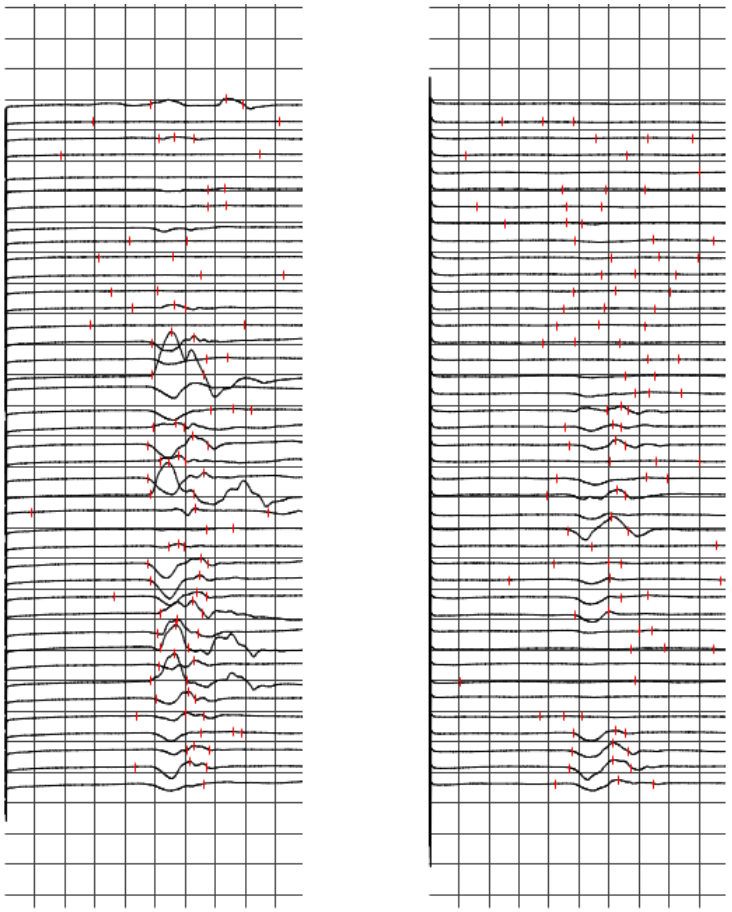
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							27.5		
Median F-Response Right									
Wrist - APB							27.9		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	--	--	
		Left-Right	0	0	



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Patient Data

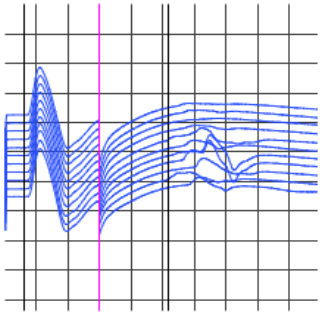
Patient ID: IBMS_A_Follow-up#2
Name:
DOB:
Age:
Sex: Female
Height:

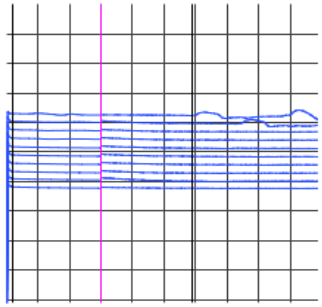
Date of Study: 09/08/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							27.5		
Wrist - APB							25.9		
Median F-Response Right									
Wrist - APB							29.5		

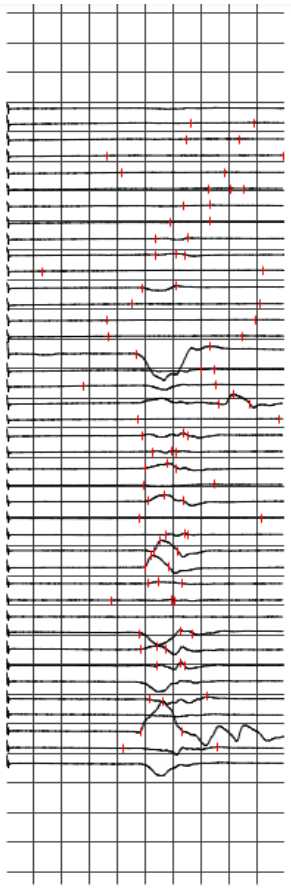
F-Wave

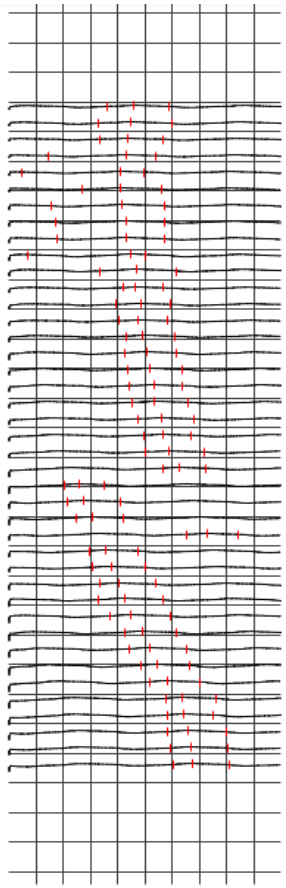




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	24.5	0.35	
		Right	24.5	0.71	
		Left-Right	0	0.36	





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Patient Data

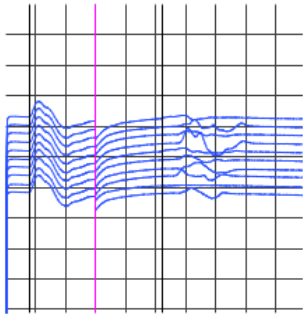
Patient ID: IBMS_A_Follow-up#3
Name:
DOB:
Age:
Sex: Female
Height:

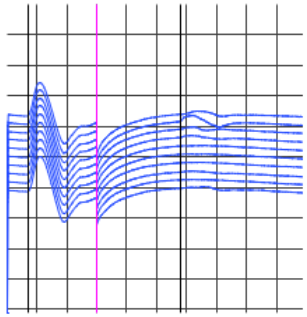
Date of Study: 6/09/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							26.1		
Median F-Response Right									
Wrist - APB							28.9		

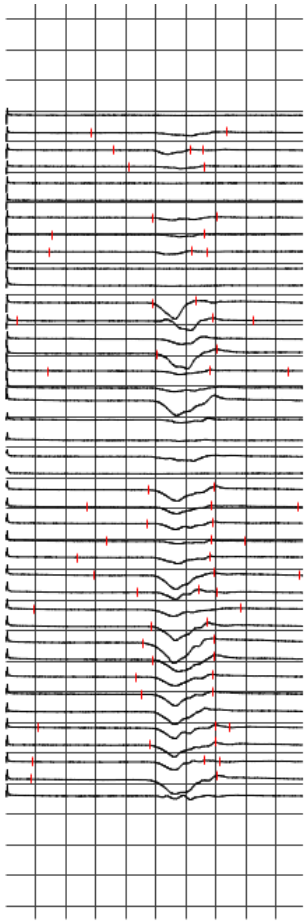
F-Wave

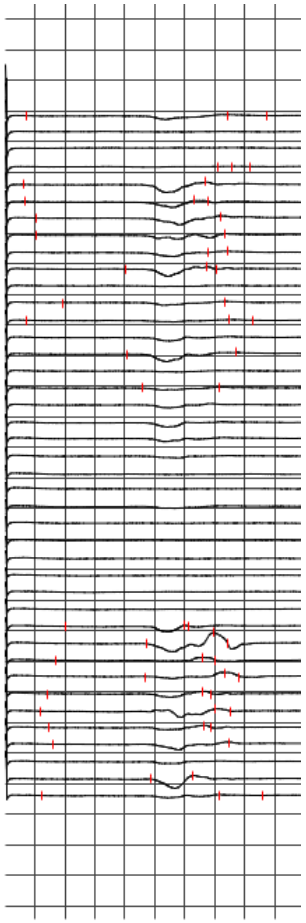




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	25.0	0	
		Right	34.3	0.075	
		Left-Right	9.3	0.075	





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Patient Data

Patient ID: IBMS_B_Baseline#1
Name:
DOB:
Age:
Sex: Female
Height:

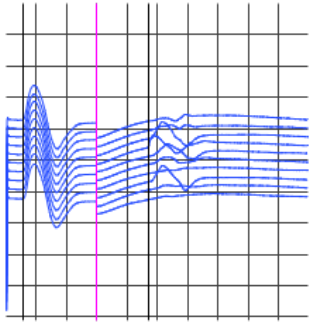
Date of Study: 08/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

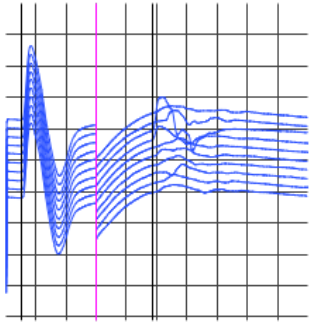
Nerve Conduction Studies - Motor

Motor NCS

Nerve	Lat (ms)	Lat ref limit	Ampl (mV)	Ampl ref limit	CV (m/s)	CV ref limit	F Lat (ms)	Distance (mm)	Limb Temp (°C)
Median F-Response Left									
Wrist - APB							23.6		
Median F-Response Right									
Wrist - APB							24.4		

F-Wave

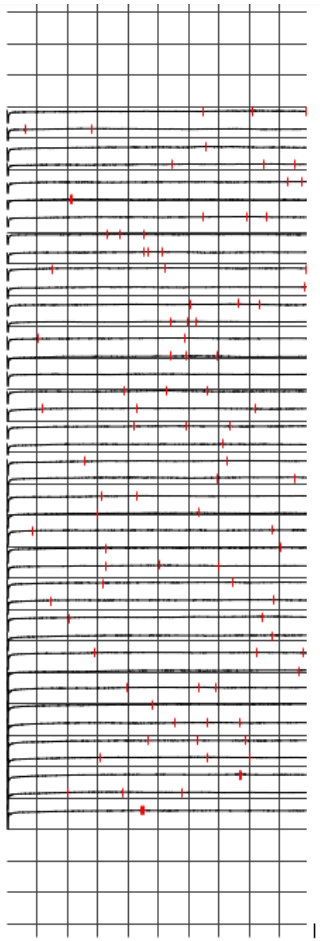


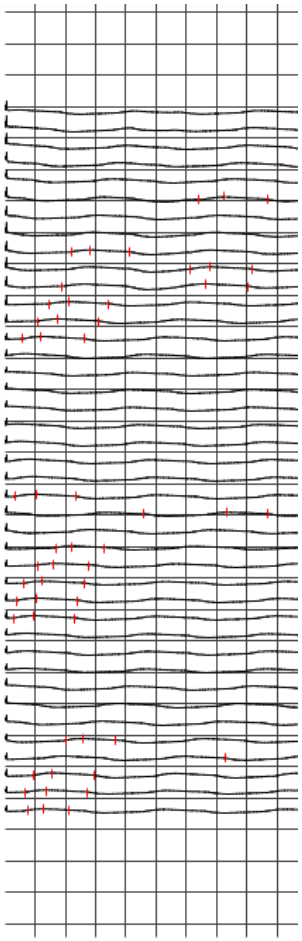


MEP Cortical/F

MEP

Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	8.2	0.029	
		Right	--	--	
		Left-Right	8.2	0.029	





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Patient Data

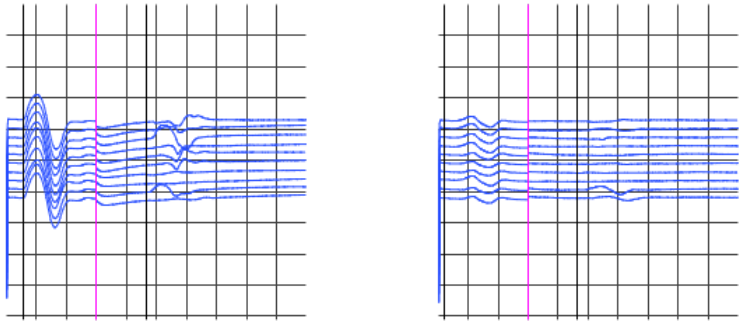
Patient ID: IBMS_B_Baseline#2
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 15/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

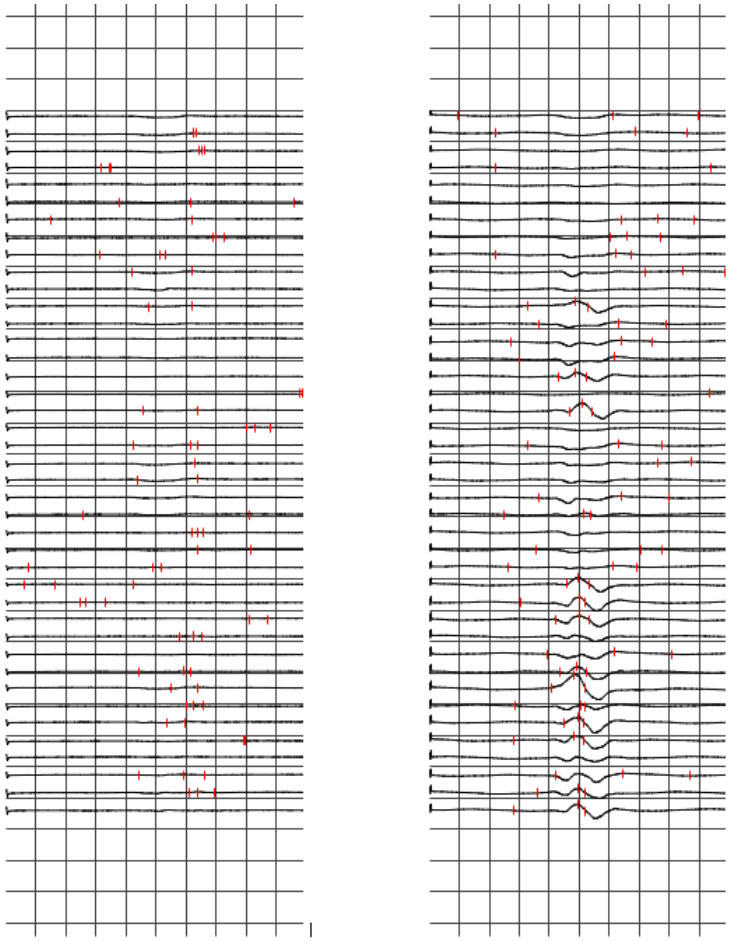
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							23.3		
Median F-Response Right									
Wrist - APB							23.2		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	29.6	0.11	
		Right	10.5	0.075	
		Left-Right	19.1	0.035	



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Patient Data

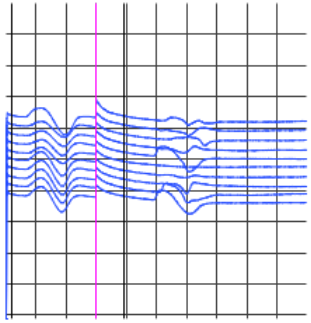
Patient ID: IBMS_B_Baseline#3
Name:
DOB:
Age:
Sex: Female
Height:

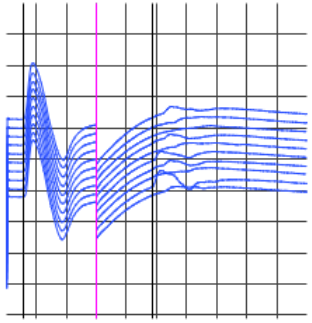
Date of Study: 22/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							19.6		
Median F-Response Right									
Wrist - APB							24.4		

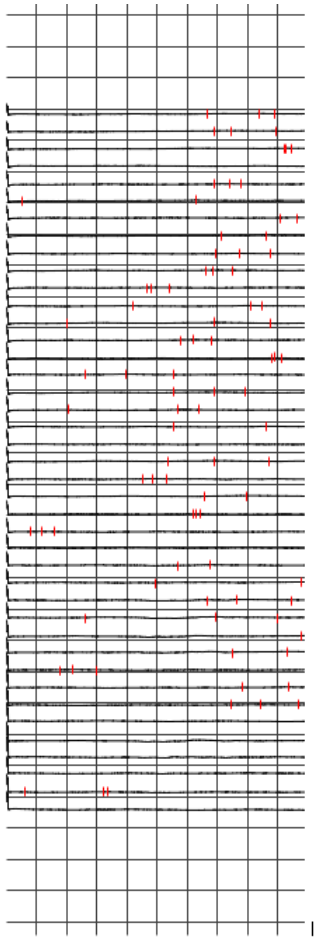
F-Wave

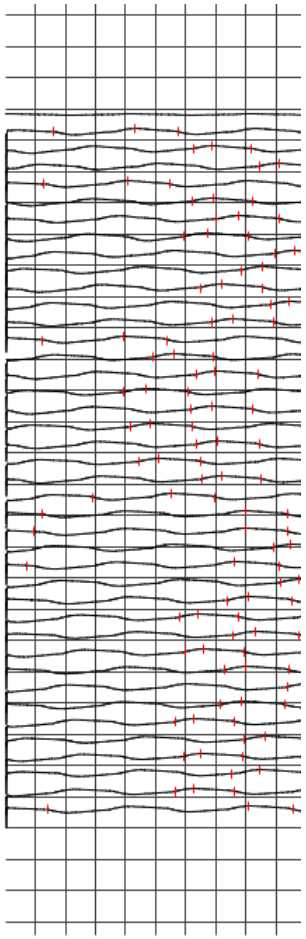




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	39.4	0.011	
		Right	--	--	
		Left-Right	39.4	0.011	





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Patient Data

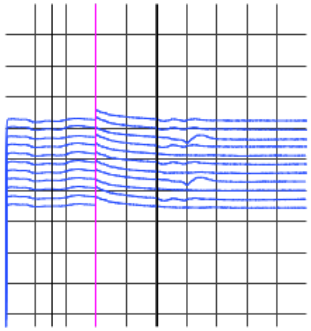
Patient ID: IBMS_B_Baseline#4
Name:
DOB:
Age:
Sex: Female
Height:

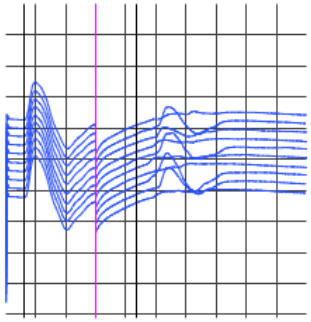
Date of Study: 29/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							25.3		
Median F-Response Right									
Wrist - APB							21.8		

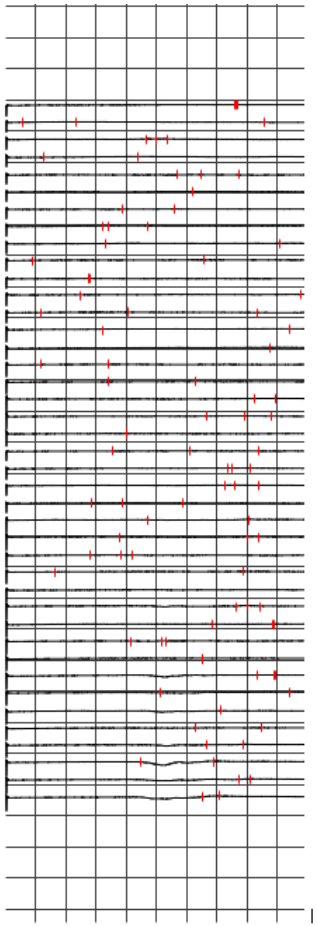
F-Wave

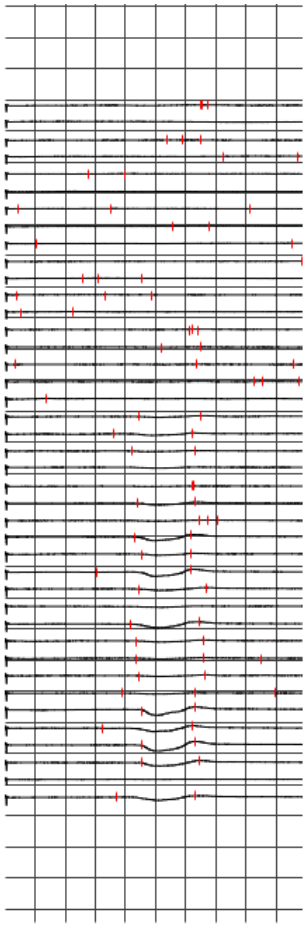




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	OCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	23.1	0.017	
		Right	--	--	
		Left-Right	23.1	0.017	





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Patient Data

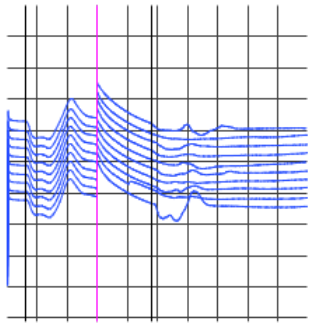
Patient ID: IBMS_B_Intervention#1
Name:
DOB:
Age:
Sex: Female
Height:

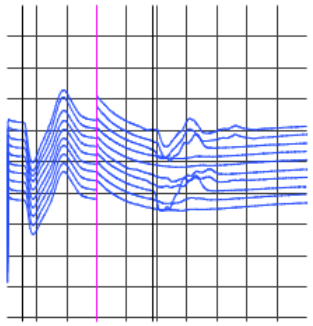
Date of Study: 24/04/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							24.0		
Median F-Response Right									
Wrist - APB							24.4		

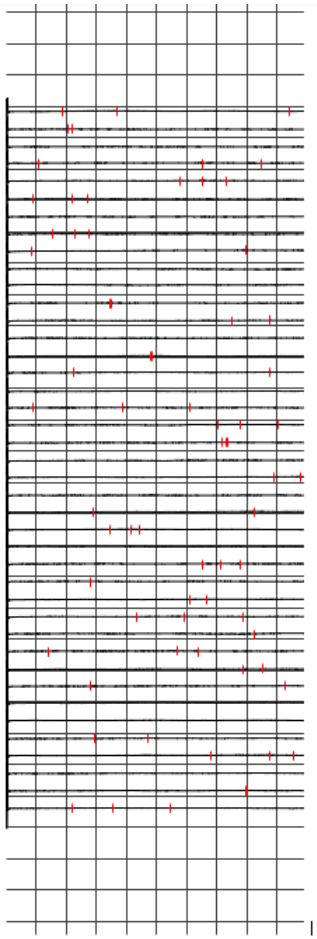
F-Wave

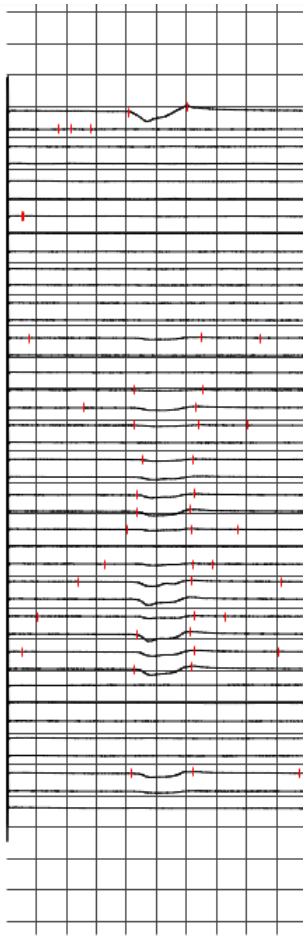




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	21.9	0.12	
		Left-Right	21.9	0.12	





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Patient Data

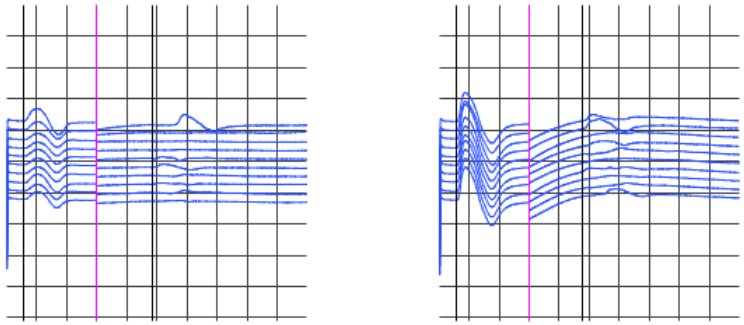
Patient ID: IBMS_B_Intervention#2
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 10/05/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

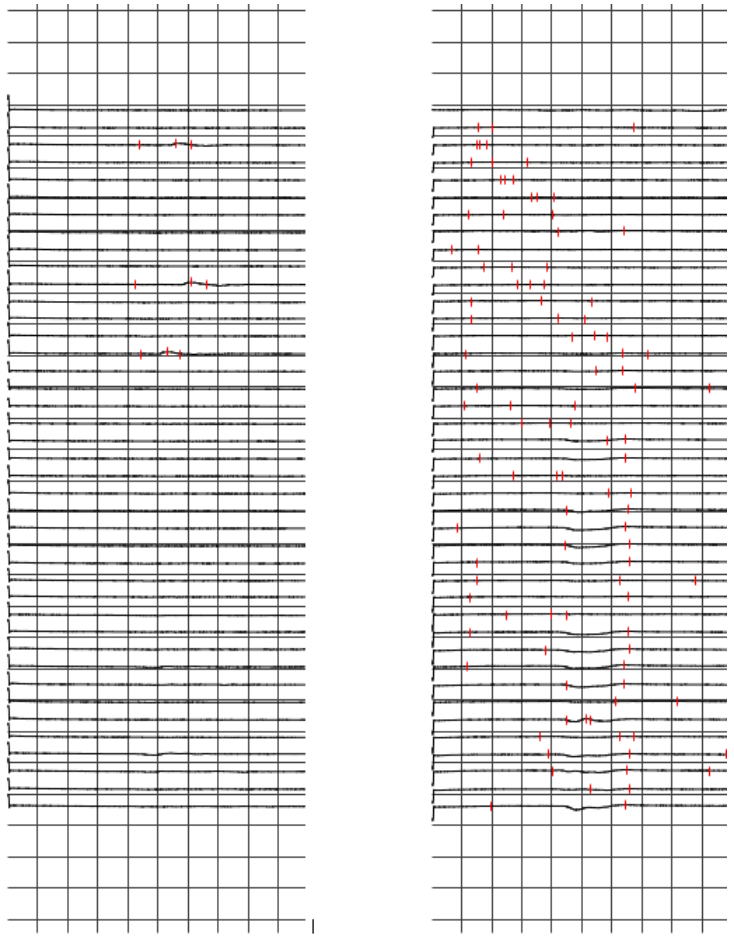
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							24.3		
Median F-Response Right									
Wrist - APB							24.0		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	--	--	
		Left-Right	0	0	



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Patient Data

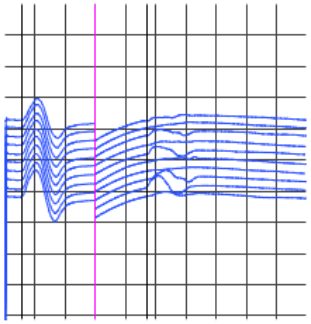
Patient ID: IBMS_B_Intervention#3
Name:
DOB:
Age:
Sex: Female
Height:

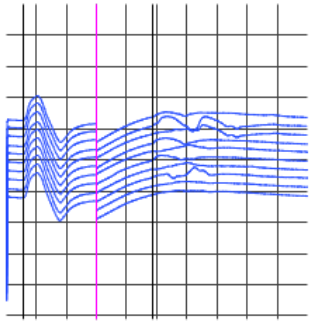
Date of Study: 24/05/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							23.6		
Median F-Response Right									
Wrist - APB							24.3		

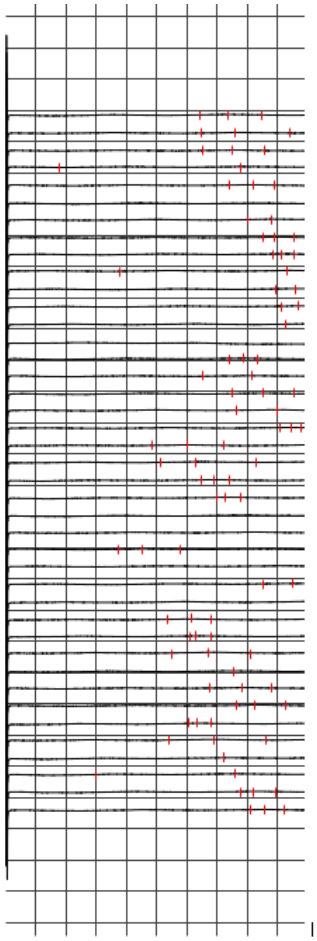
F-Wave

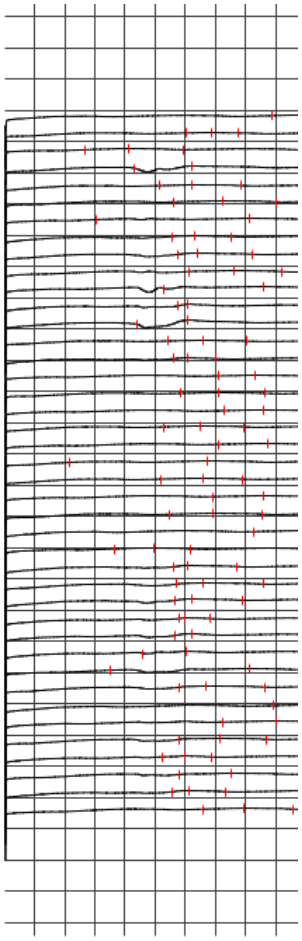




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	--	--	
		Right	--	--	
		Left-Right	0	0	





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Patient Data

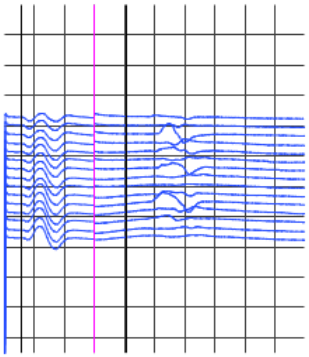
Patient ID: IBMS_B_Intervention#4
Name:
DOB:
Age:
Sex: Female
Height:

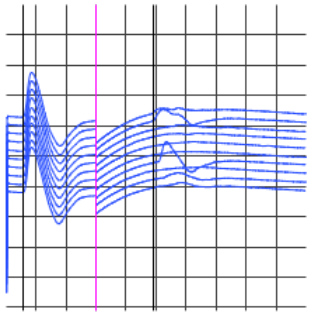
Date of Study: 07/06/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							20.3		
Median F-Response Right									
Wrist - APB							24.5		

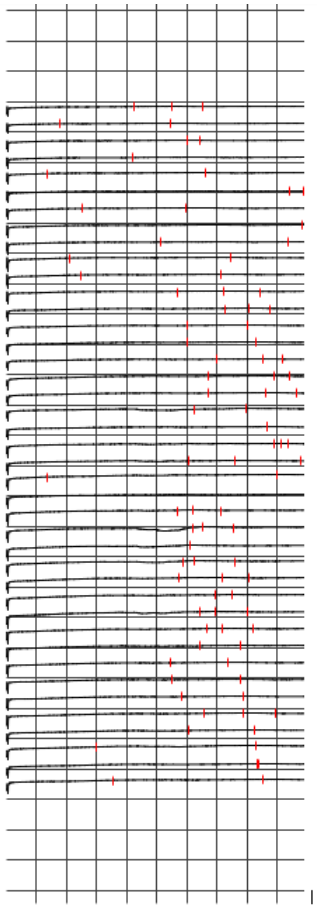
F-Wave

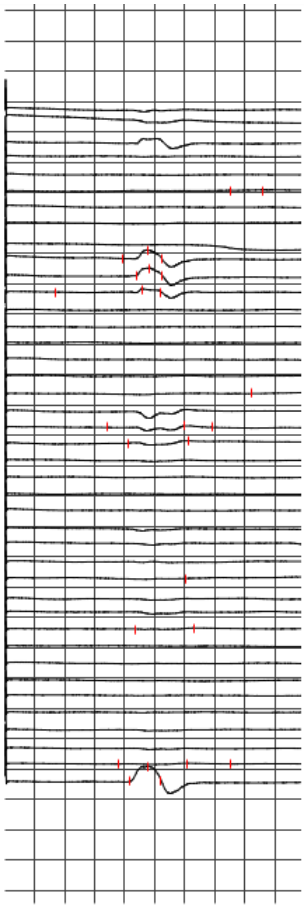




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	--	--	
		Right	--	--	
		Left-Right	0	0	





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Patient Data

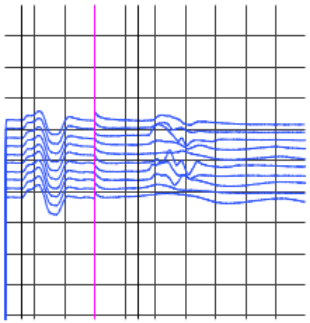
Patient ID: IBMS_B_Intervention#5
Name:
DOB:
Age:
Sex: Female
Height:

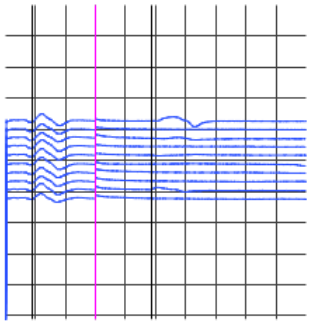
Date of Study: 21/06/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							22.2		
Median F-Response Right									
Wrist - APB							24.4		

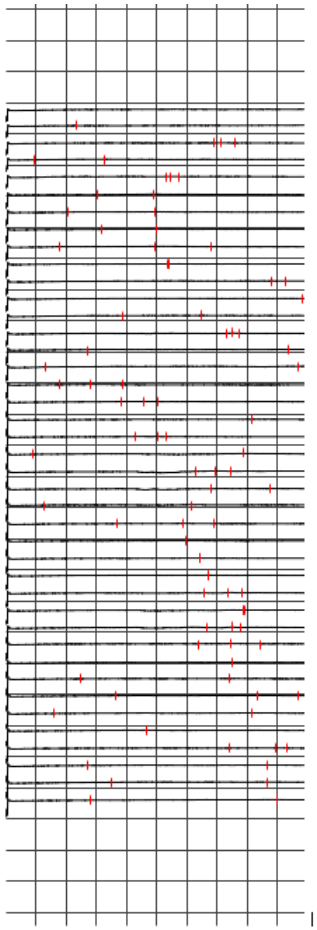
F-Wave

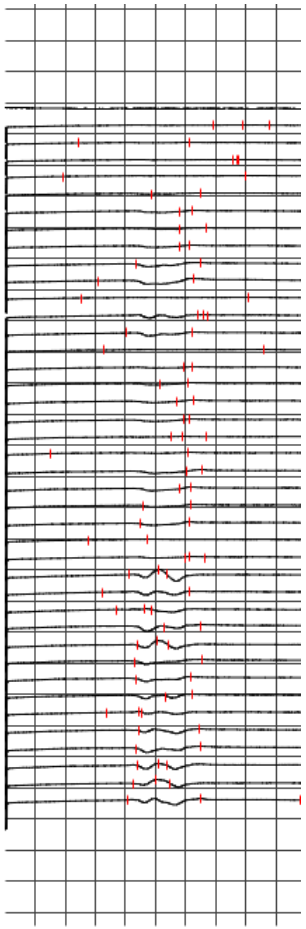




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	--	--	
		Right	--	--	
		Left-Right	0	0	





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Patient Data

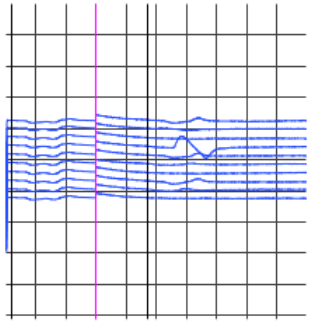
Patient ID: IBMS_B_Follow-up#1
Name:
DOB:
Age:
Sex: Female
Height:

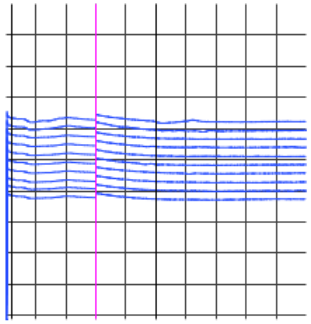
Date of Study: 19/07/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							23.7		
Median F-Response Right									
Wrist - APB							25.0		

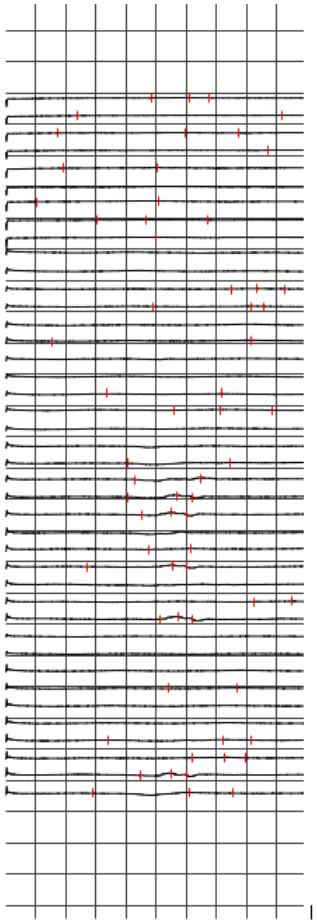
F-Wave

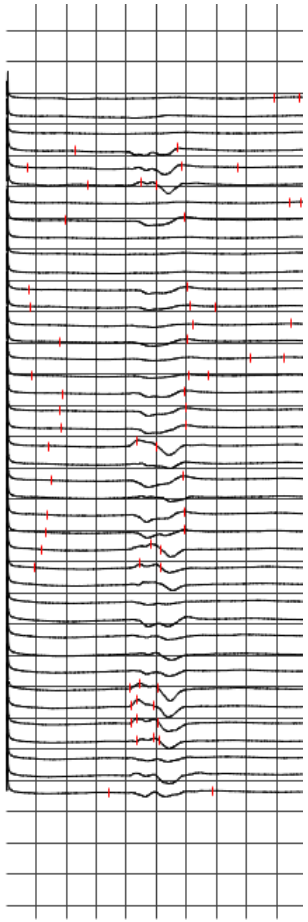




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	15.0	0.023	
		Left-Right	15.0	0.023	





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Patient Data

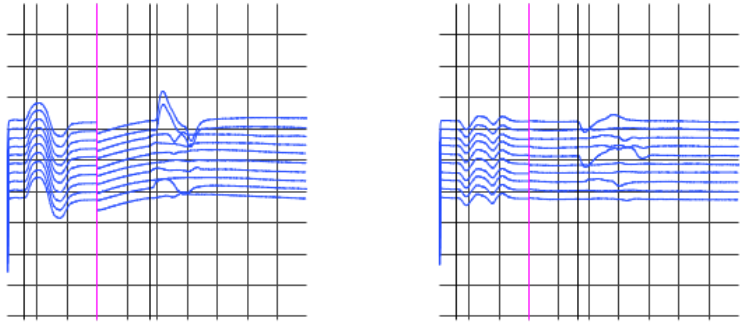
Patient ID: IBMS_B_Follow-up#2
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 18/08/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

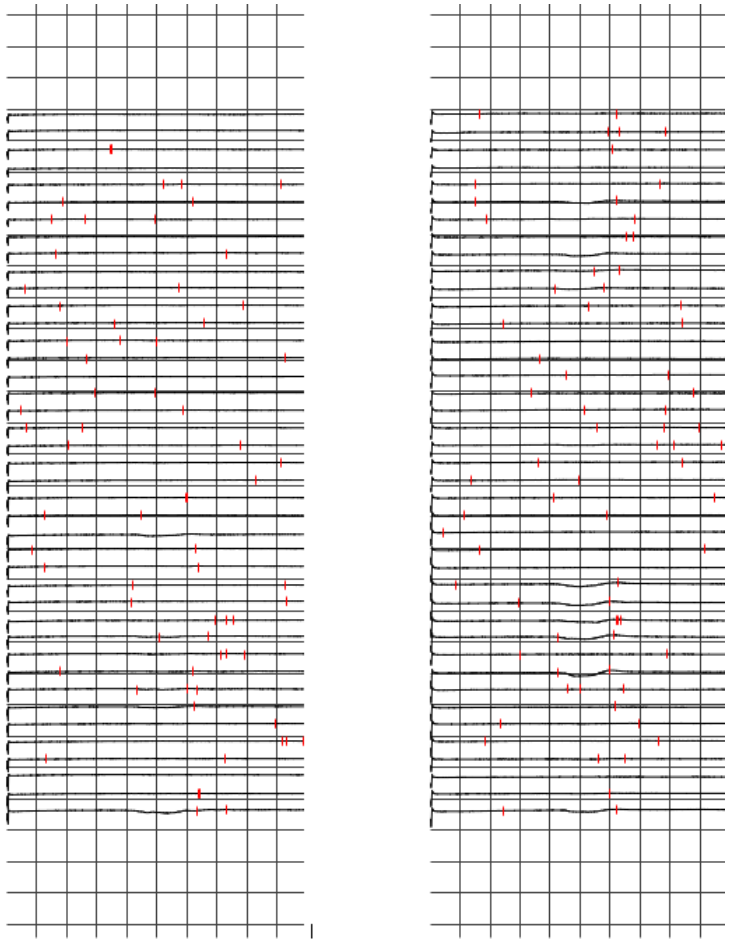
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							23.8		
Median F-Response Right									
Wrist - APB							23.3		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	16.5	0.067	
		Left-Right	16.5	0.067	



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Patient Data

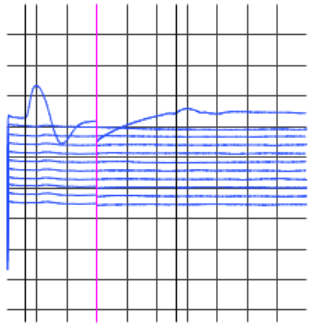
Patient ID: IBMS_B_Foloow-up#3
Name:
DOB:
Age:
Sex: Female
Height:

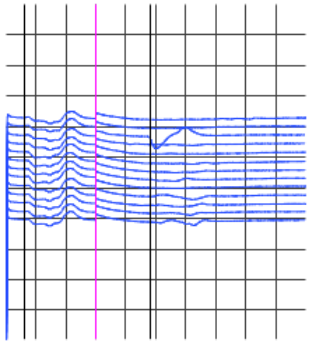
Date of Study: 13/09/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							28.3		
Median F-Response Right									
Wrist - APB							24.1		

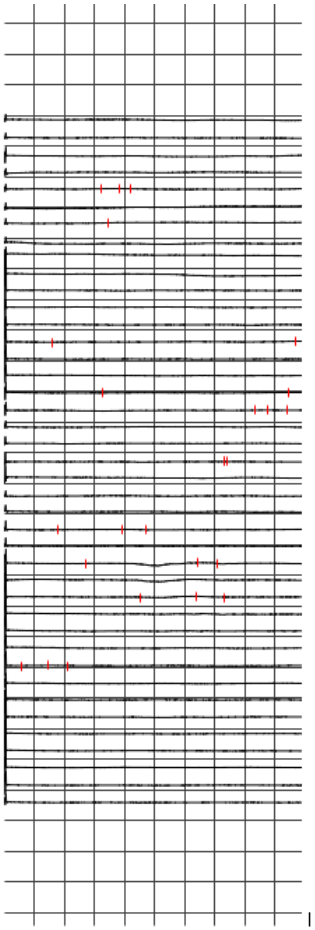
F-Wave

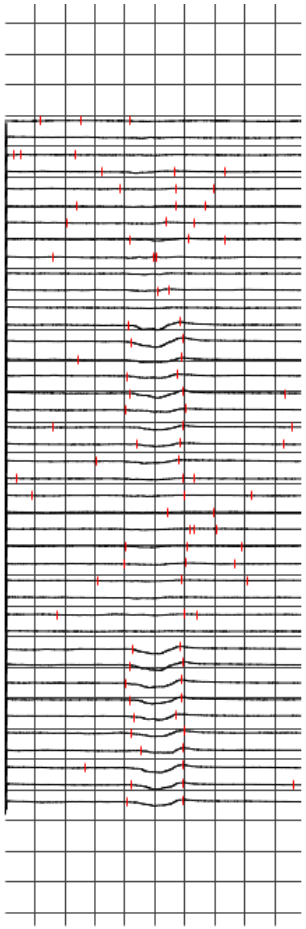




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	--	--	
		Right	--	--	
		Left-Right	0	0	





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Patient Data

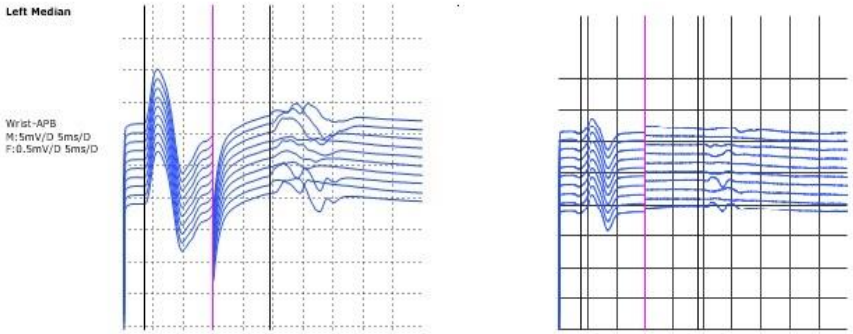
Patient ID: IBMS_C_Baseline#1
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 10/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

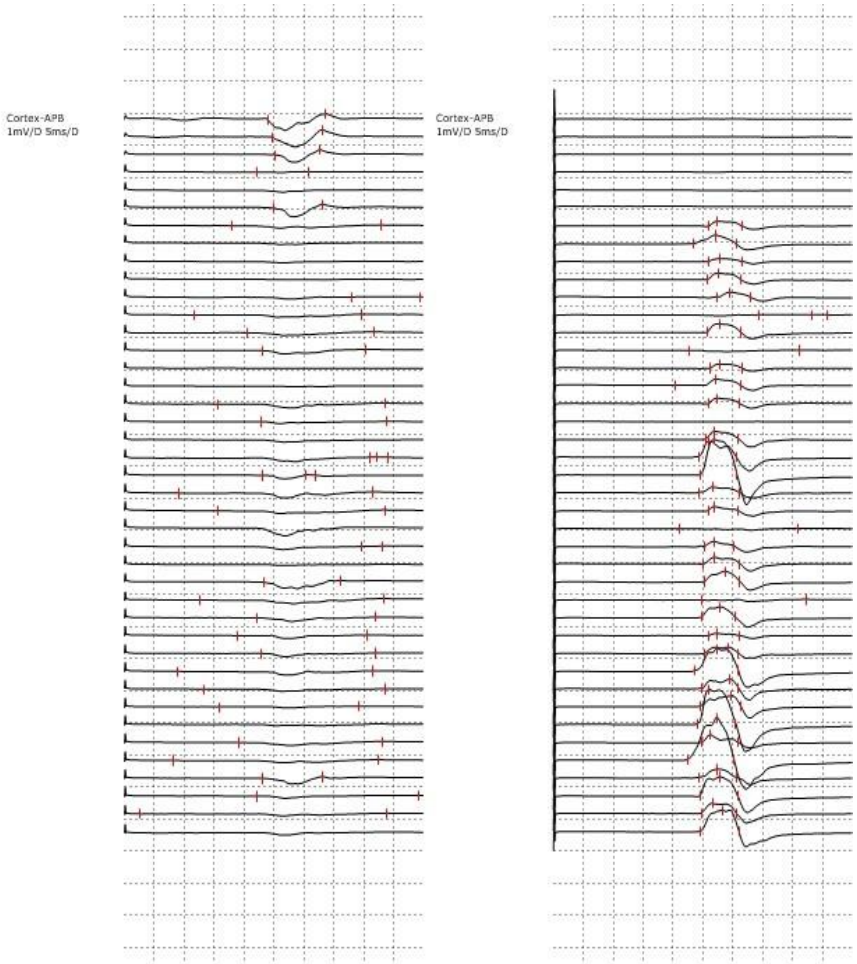
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							24.5		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	22.0	0.012	
		Right	24.6	0.58	
		Left-Right	2.6	0.57	



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Patient Data

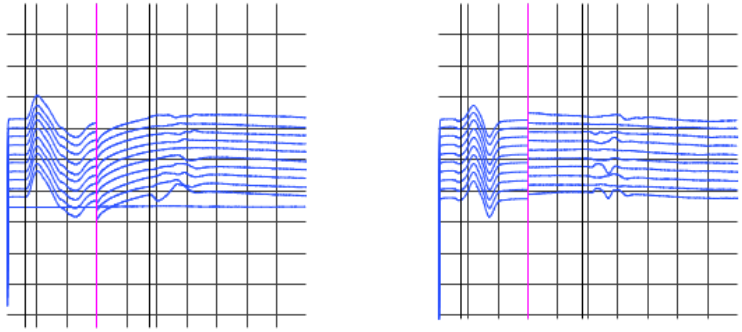
Patient ID: IBMS_C_Baseline#2
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 17/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

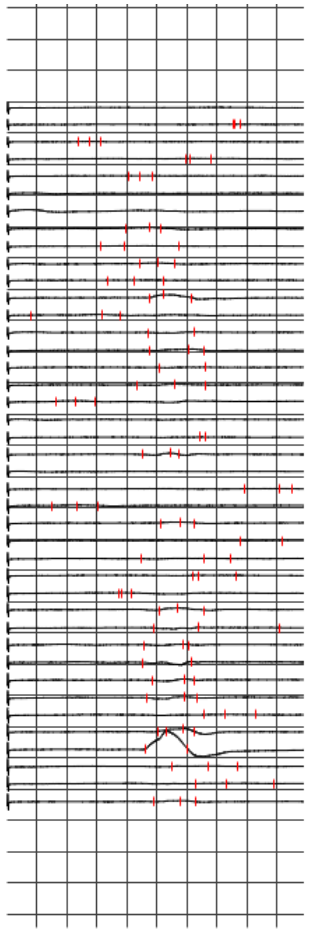
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							23.7		
Median F-Response Right									
Wrist - APB							24.1		

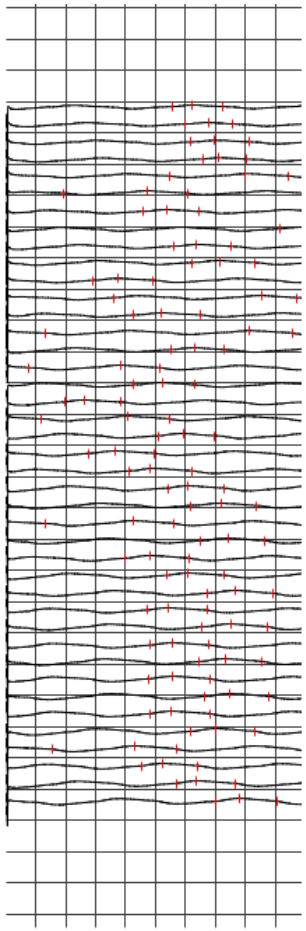
F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	22.3	0.036	
		Right	32.7	0.092	
		Left-Right	10.4	0.056	





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Patient Data

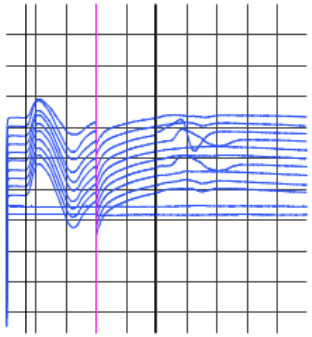
Patient ID: IBMS_C-Baseline#3
Name:
DOB:
Age:
Sex: Female
Height:

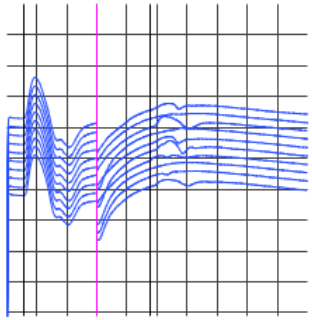
Date of Study: 24/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							24.6		
Median F-Response Right									
Wrist - APB							23.9		

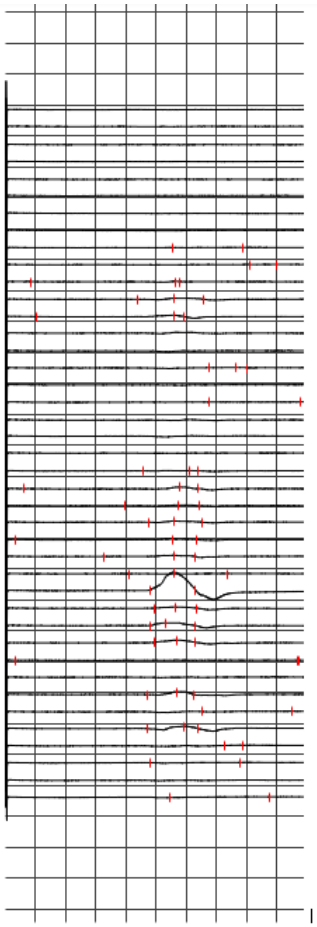
F-Wave

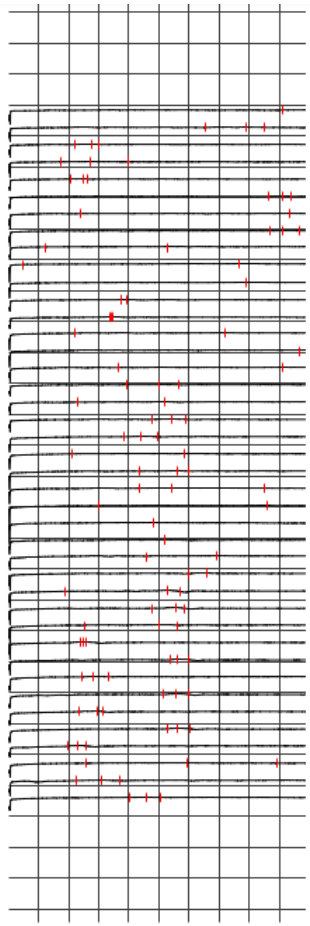




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	32.9	0.050	
		Right	25.5	0.10	
		Left-Right	7.4	0.050	





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Patient Data

Patient ID: IBMS_C_Baseline#4
Name:
DOB:
Age:
Sex: Female
Height:

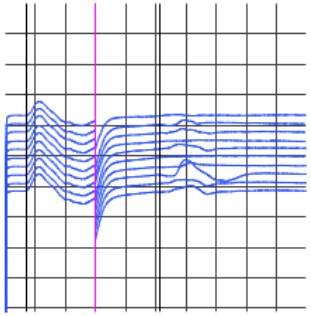
Date of Study: 31/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

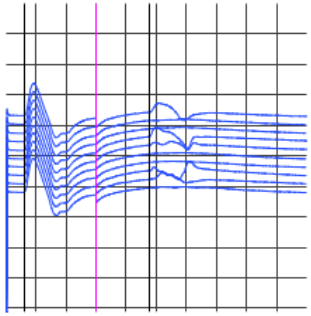
Nerve Conduction Studies - Motor

Motor NCS

Nerve	Lat (ms)	Lat ref limit	Ampl (mV)	Ampl ref limit	CV (m/s)	CV ref limit	F Lat (ms)	Distance (mm)	Limb Temp (°C)
Median F-Response Left									
Wrist - APB							25.7		
Median F-Response Right									
Wrist - APB							24.0		

F-Wave

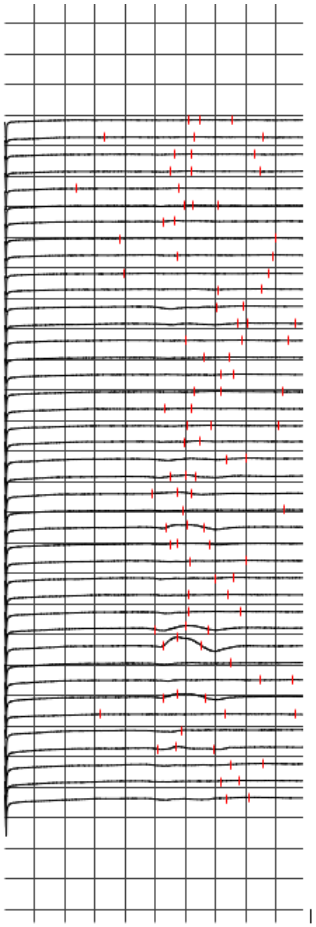


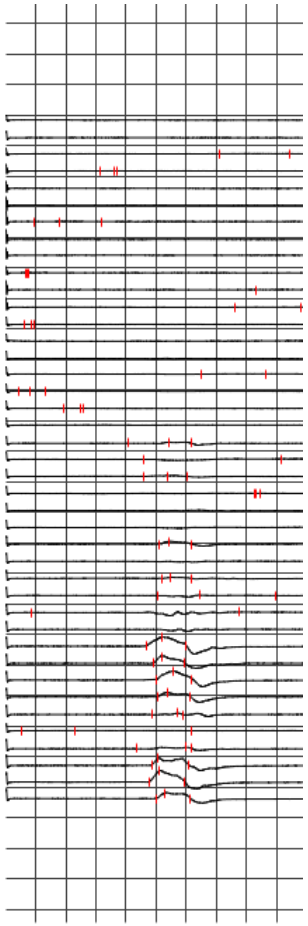


MEP Cortical/F

MEP

Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	37.2	0.085	
		Right	24.0	0.29	
		Left-Right	13.2	0.21	





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Patient Data

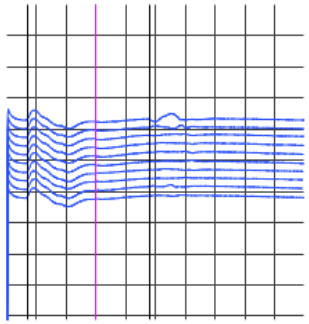
Patient ID: IBMS_C_Baseline#5
Name:
DOB:
Age:
Sex: Female
Height:

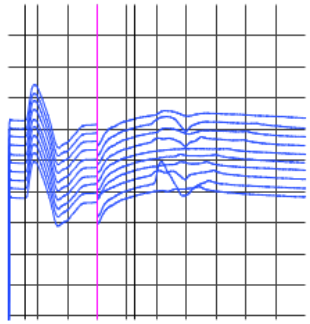
Date of Study: 07/04/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							24.0		
Median F-Response Right									
Wrist - APB							21.3		

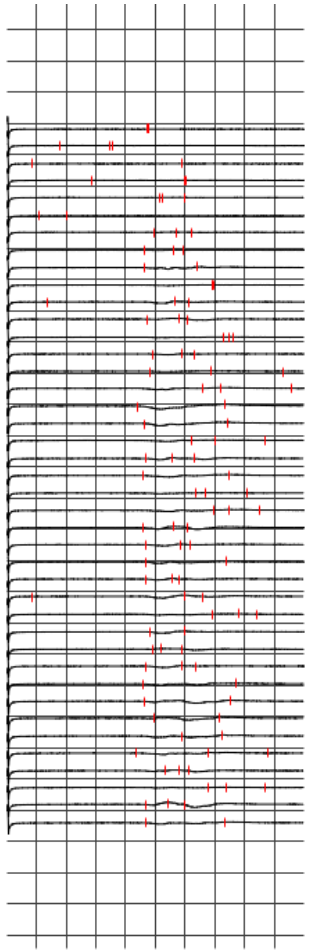
F-Wave

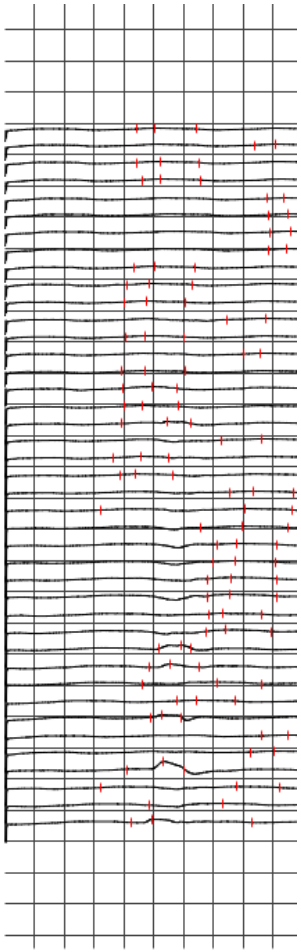




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	--	--	
		Right	--	--	
		Left-Right	0	0	





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Patient Data

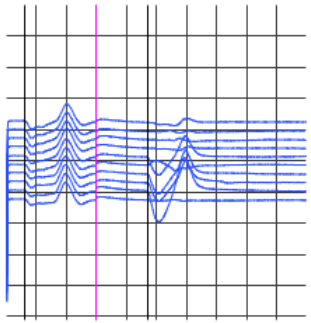
Patient ID: IBMS_C_Intervention#1
Name:
DOB:
Age:
Sex: Female
Height:

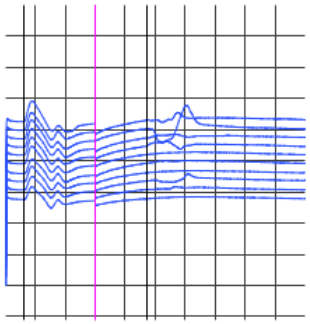
Date of Study: 5/05/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							23.7		
Median F-Response Right									
Wrist - APB							23.6		

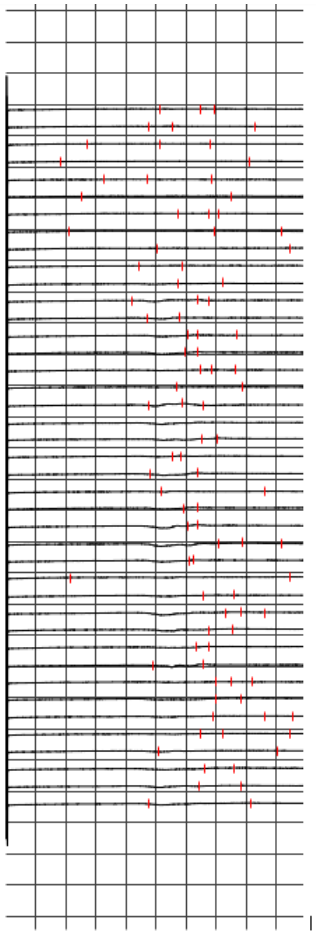
F-Wave

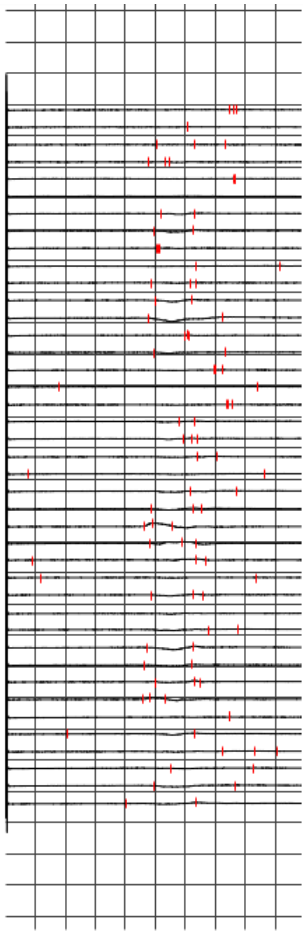




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	15.3	0	
		Right	41.3	0	
		Left-Right	26.0	0	





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Patient Data

Patient ID: IBMS_C_Intervention#2
Name:
DOB:
Age:
Sex: Female
Height:

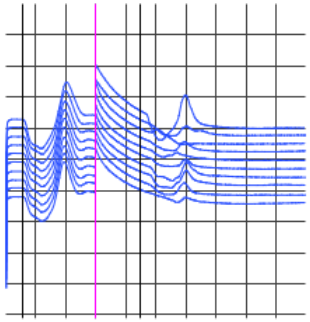
Date of Study: 17/05/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

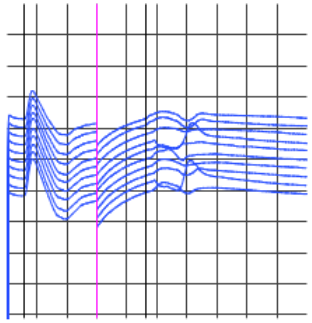
Nerve Conduction Studies - Motor

Motor NCS

Nerve	Lat (ms)	Lat ref limit	Ampl (mV)	Ampl ref limit	CV (m/s)	CV ref limit	F Lat (ms)	Distance (mm)	Limb Temp (°C)
Median F-Response Left									
Wrist - APB							22.5		
Median F-Response Right									
Wrist - APB							23.3		

F-Wave

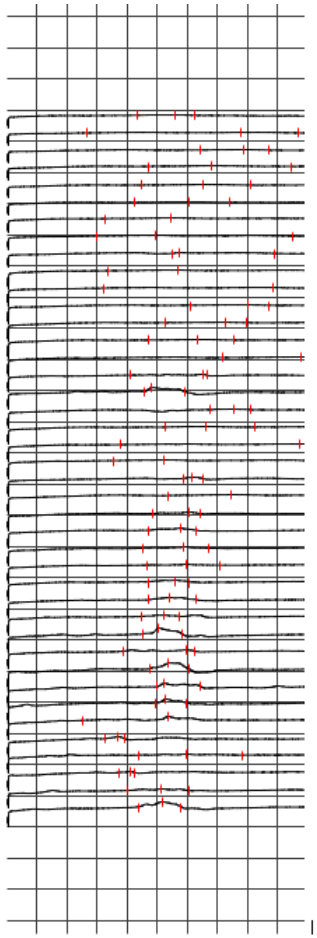


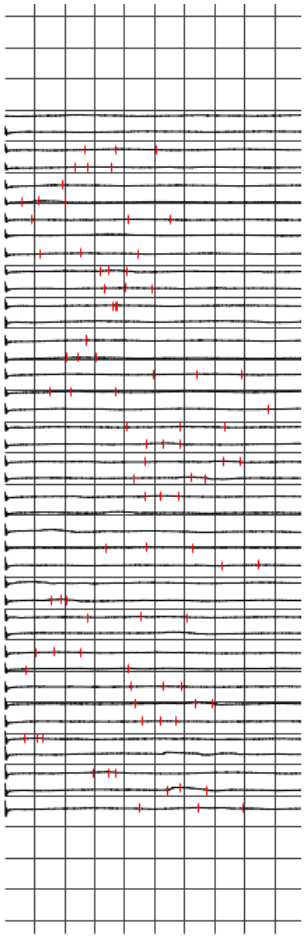


MEP Cortical/F

MEP

Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	20.6	0.26	
		Right	25.3	0.22	
		Left-Right	4.7	0.040	





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Patient Data

Patient ID: IBMS_C_Intervention#3
Name:
DOB:
Age:
Sex: Female
Height:

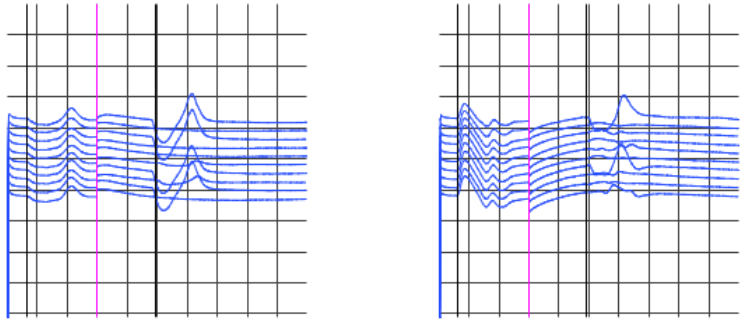
Date of Study: 2/06/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS

Nerve	Lat (ms)	Lat ref limit	Ampl (mV)	Ampl ref limit	CV (m/s)	CV ref limit	F Lat (ms)	Distance (mm)	Limb Temp (°C)
Median F-Response Left									
Wrist - APB							24.8		
Median F-Response Right									
Wrist - APB							24.6		

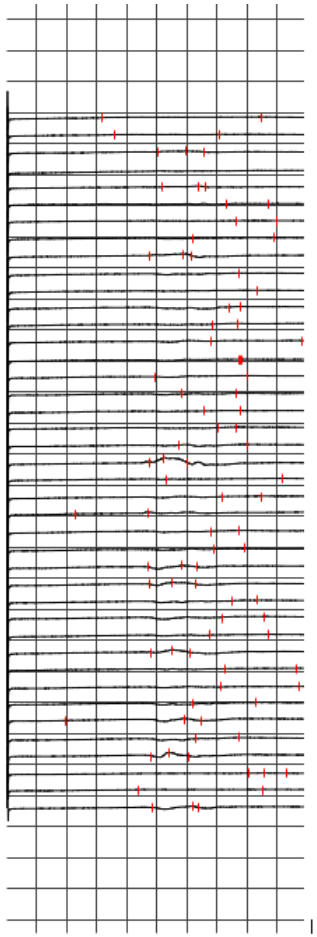
F-Wave

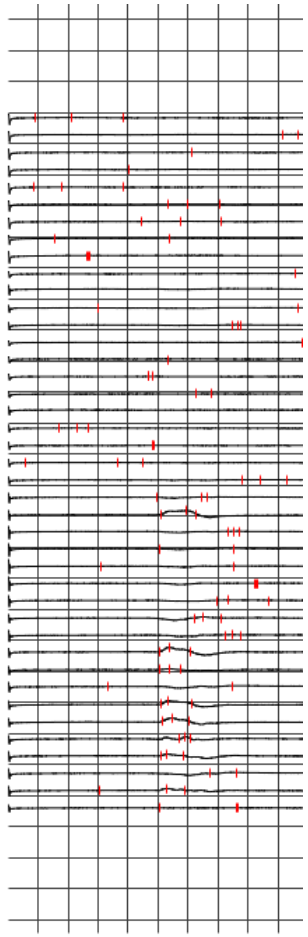


MEP Cortical/F

MEP

Nerve	Site	Side	Lat ms	Amp mV	OCT ms
Ulnar	Cortex - APB	Left	--	--	
		Right	22.8	0.052	
		Left-Right	22.8	0.052	





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Patient Data

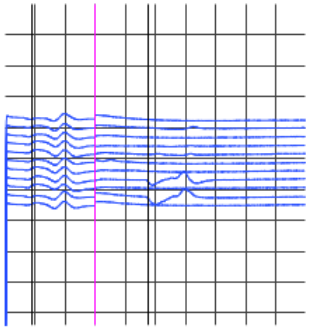
Patient ID: IBMS_C_Intervention#4
Name:
DOB:
Age:
Sex: Female
Height:

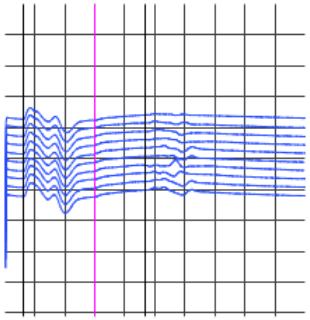
Date of Study: 14/06/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							23.8		
Median F-Response Right									
Wrist - APB							23.4		

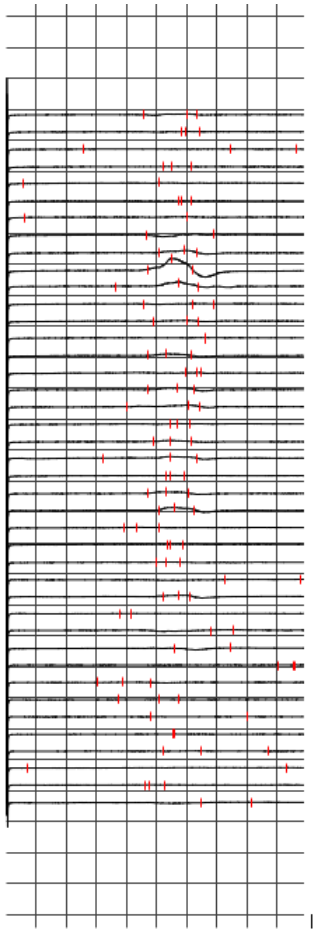
F-Wave

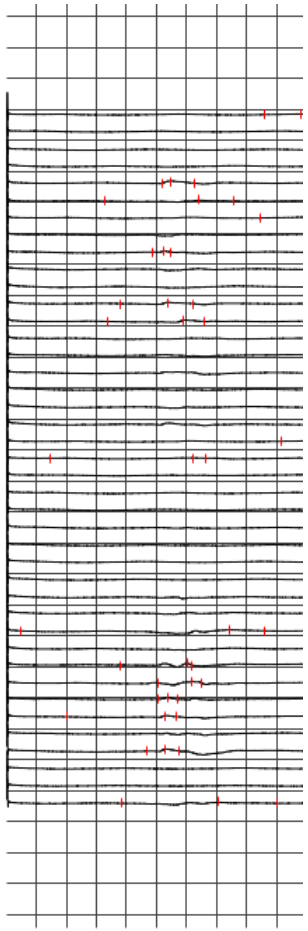




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	20.9	0	
		Right	--	--	
		Left-Right	20.9	0	





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Patient Data

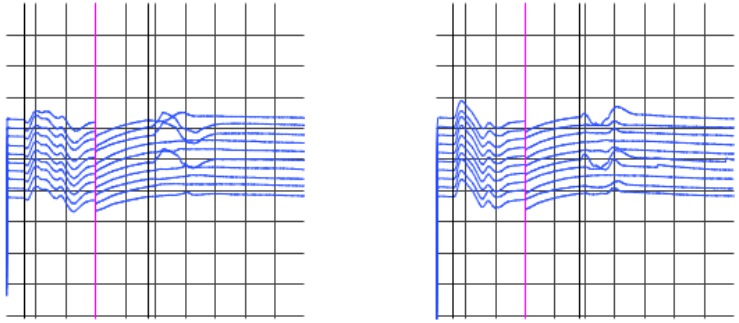
Patient ID: IBMS_C_Intervention#5
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 30/06/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

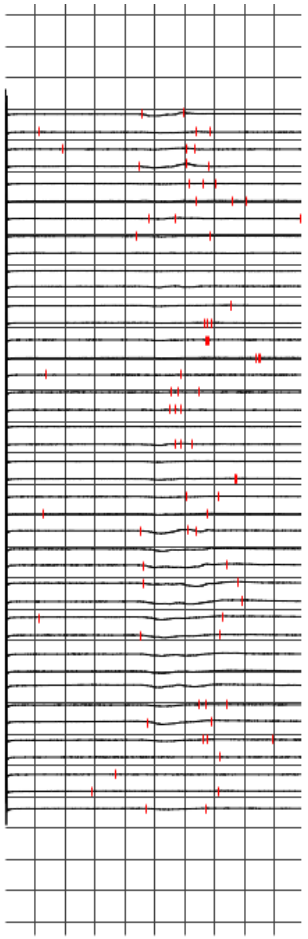
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							23.7		
Median F-Response Right									
Wrist - APB							24.1		

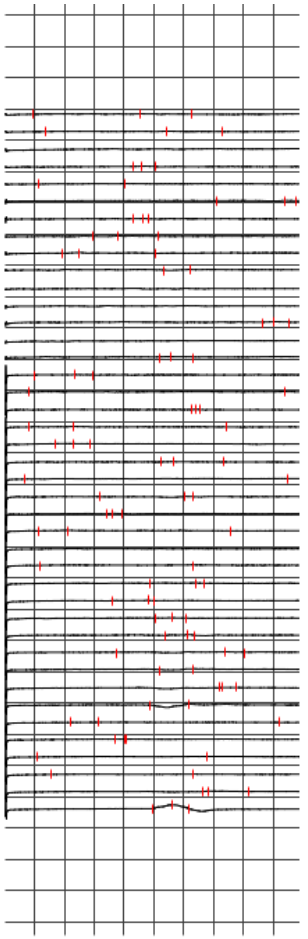
F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	42.3	0	
		Left-Right	42.3	0	





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Patient Data

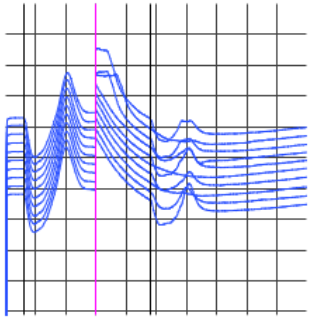
Patient ID: IBMS_C_Follow-up#1
Name:
DOB:
Age:
Sex: Female
Height:

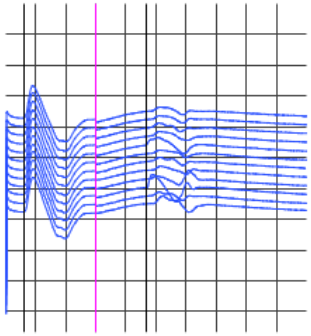
Date of Study: 28/07/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							24.1		
Median F-Response Right									
Wrist - APB							23.4		

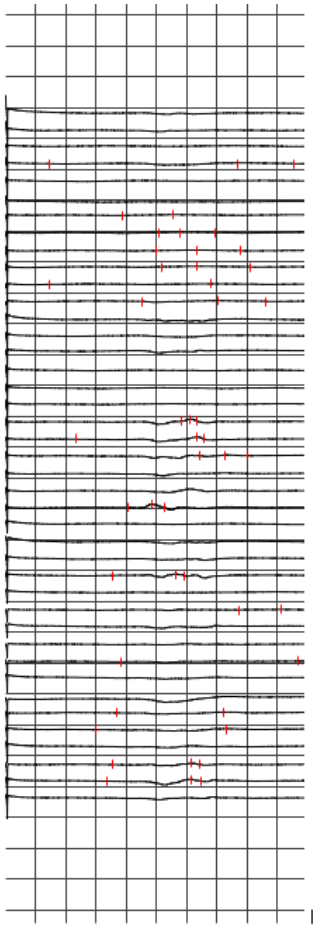
F-Wave

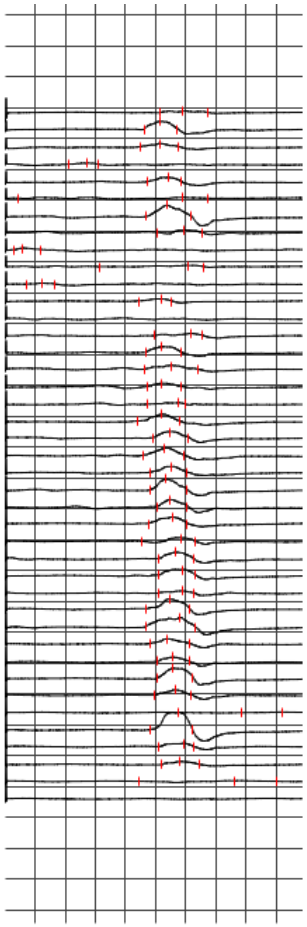




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	24.9	0.27	
		Left-Right	24.9	0.27	





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Patient Data

Patient ID: IBMS_C_Follow-up#2
Name:
DOB:
Age:
Sex: Female
Height:

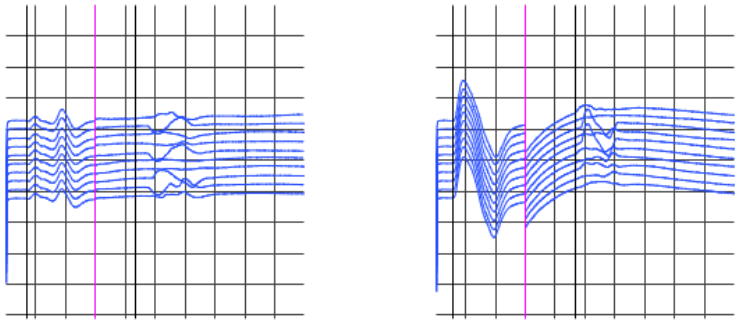
Date of Study: 25/08/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS

Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							21.7		
Median F-Response Right									
Wrist - APB							23.5		

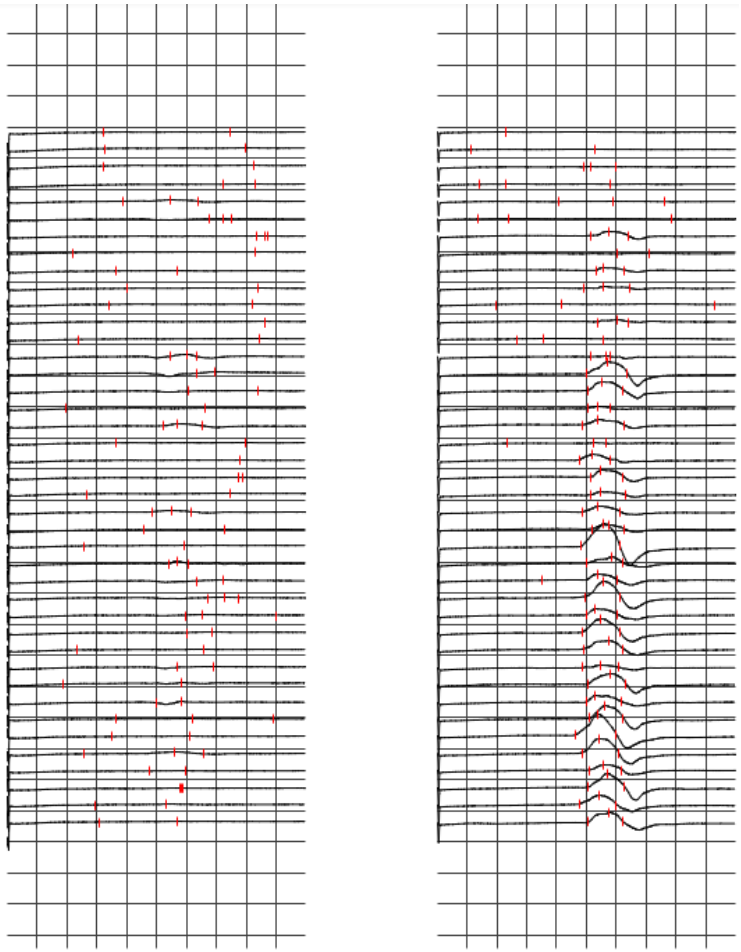
F-Wave



MEP Cortical/F

MEP

Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	9.4	0	
		Right	--	--	
		Left-Right	9.4	0	



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Patient Data

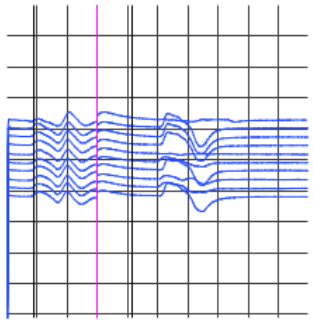
Patient ID: IBMS_C_Follow-up#3
Name:
DOB:
Age:
Sex: Female
Height:

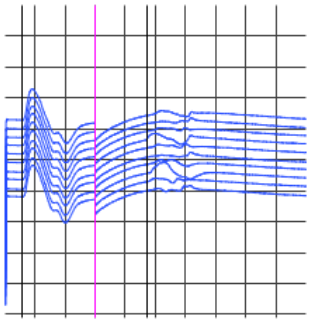
Date of Study: 20/09/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							20.7		
Median F-Response Right									
Wrist - APB							23.6		

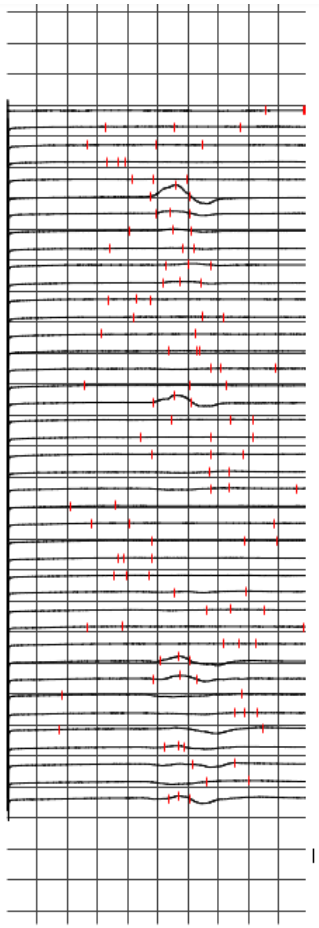
F-Wave

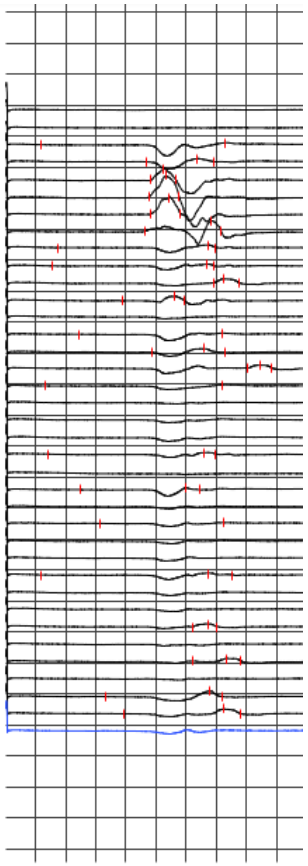




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	7.9	0.023	
		Right	--	--	
		Left-Right	7.9	0.023	





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Patient Data

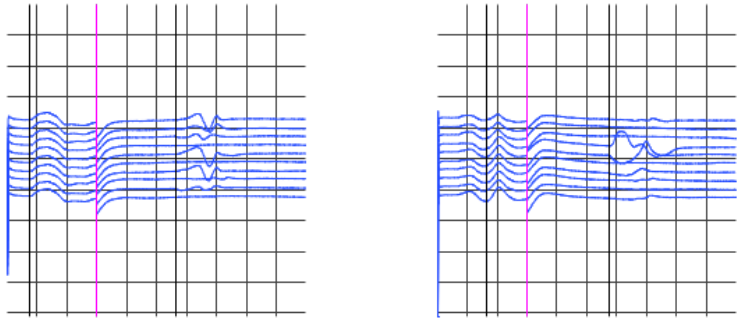
Patient ID: IBMS_D_Baseline#1
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 08/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

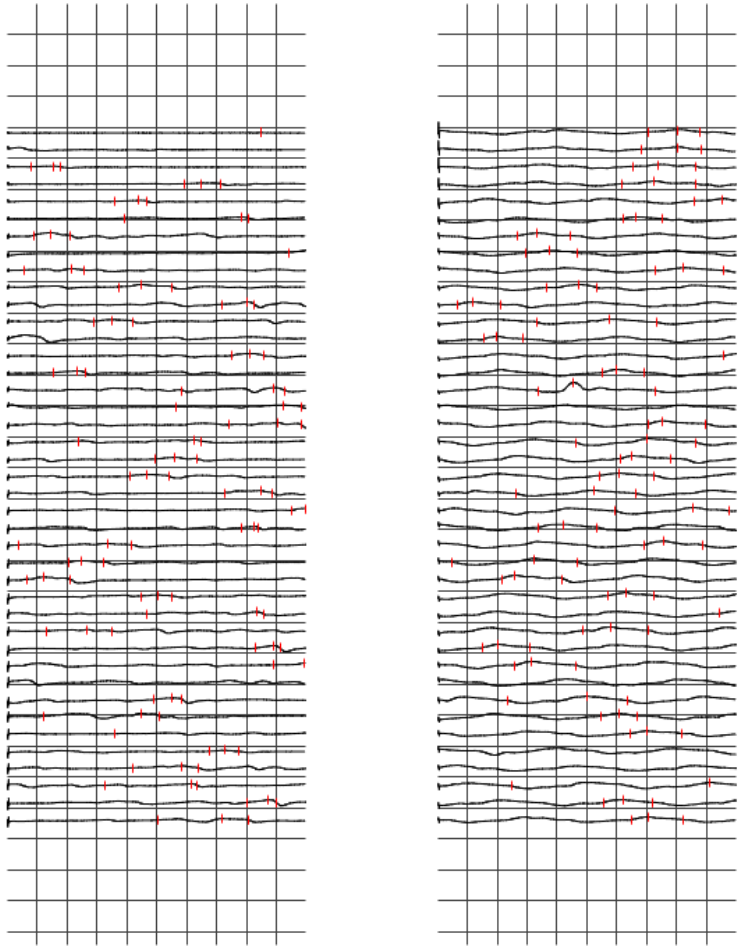
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							28.3		
Median F-Response Right									
Wrist - APB							28.7		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	26.6	0.10	
		Right	6.8	0.069	
		Left-Right	19.8	0.031	



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Patient Data

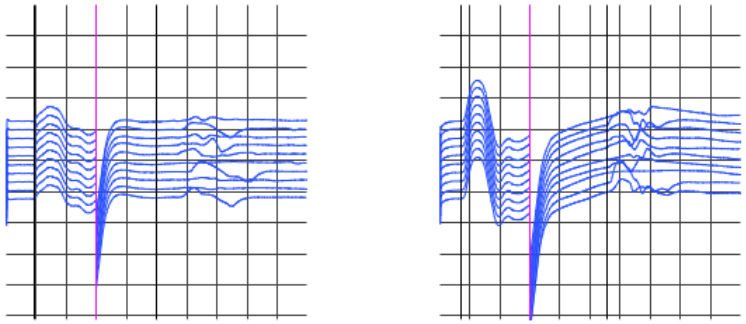
Patient ID: IBMS_D_Baseline#2
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 15/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

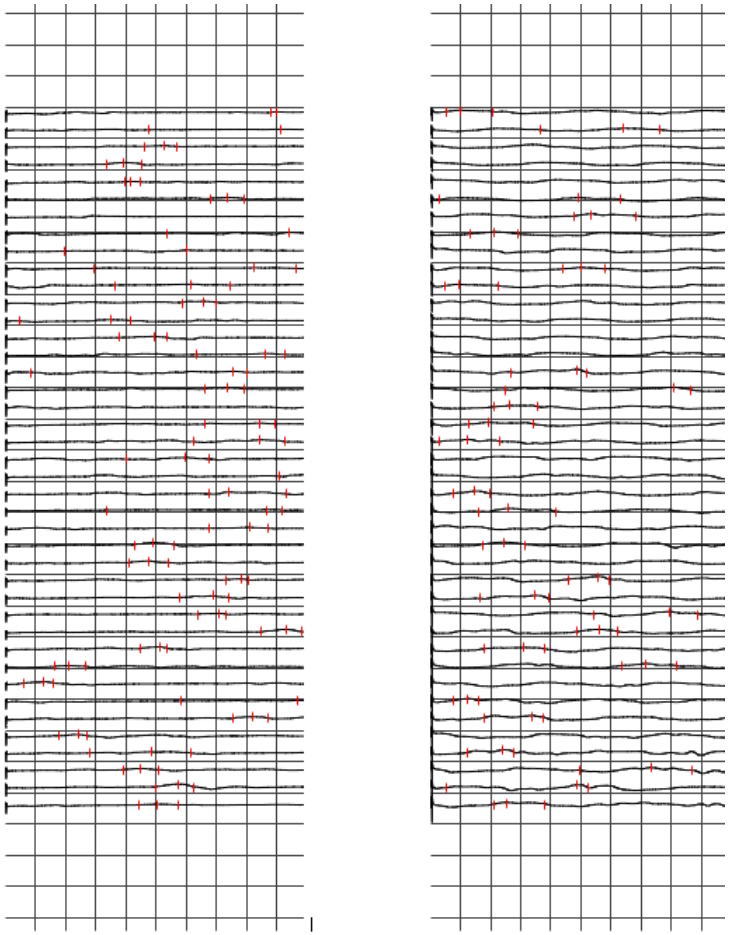
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							25.0		
Median F-Response Right									
Wrist - APB							27.8		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	30.6	0.049	
		Right	47.7	0.094	
		Left-Right	17.1	0.045	



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Patient Data

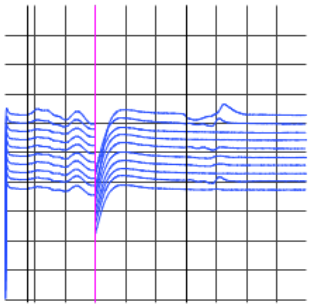
Patient ID: IBMS_D_Baseline#3
Name:
DOB:
Age:
Sex: Female
Height:

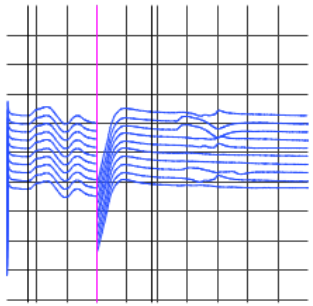
Date of Study: 22/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							24.8		
Wrist - APB							30.1		
Median F-Response Right									
Wrist - APB							24.1		

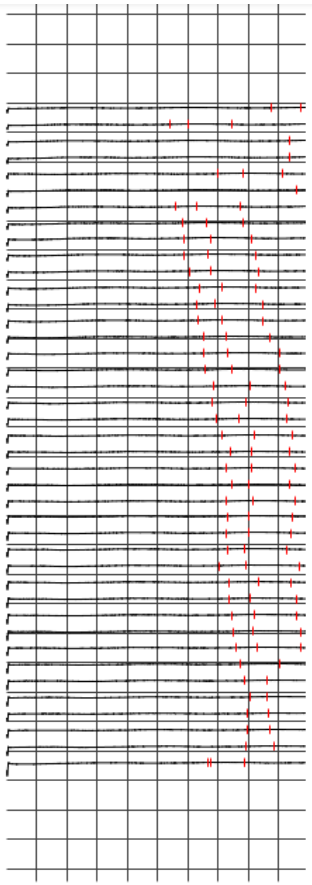
F-Wave

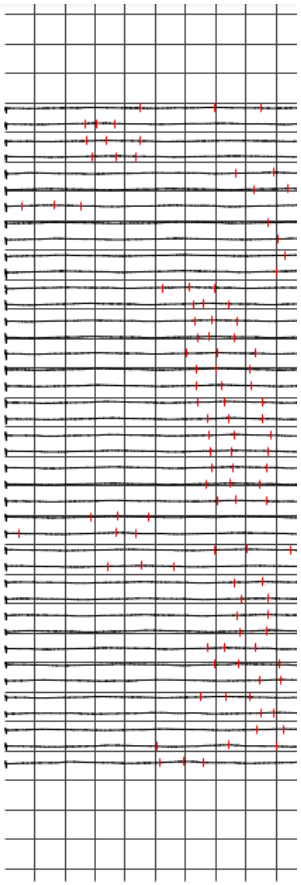




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	--	--	
		Right	3.4	0	
		Left-Right	3.4	0	





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Patient Data

Patient ID: IBMS_D_Baseline#4
Name:
DOB:
Age:
Sex: Female
Height:

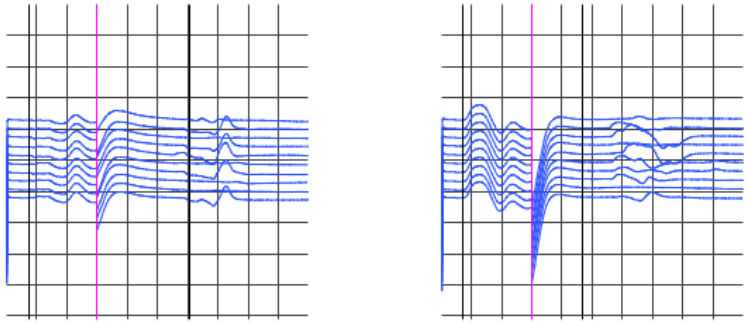
Date of Study: 29/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS

Nerve	Lat (ms)	Lat ref limit	Ampl (mV)	Ampl ref limit	CV (m/s)	CV ref limit	F Lat (ms)	Distance (mm)	Limb Temp (°C)
Median F-Response Left									
Wrist - APB							30.3		
Median F-Response Right									
Wrist - APB							23.4		

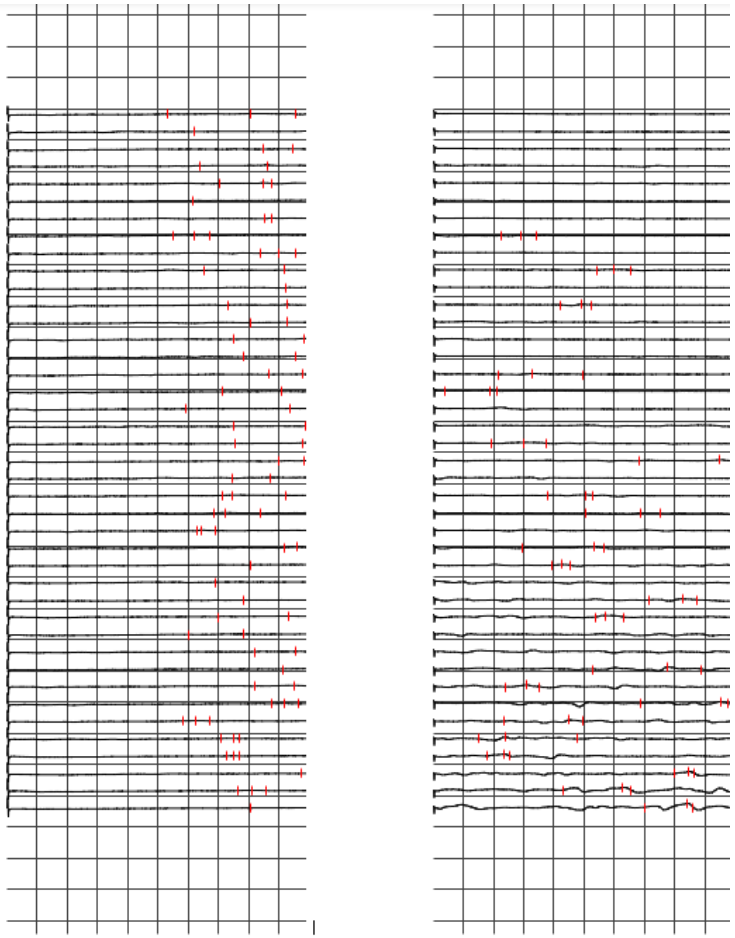
F-Wave



MEP Cortical/F

MEP

Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	--	--	
		Right	--	--	
		Left-Right	0	0	



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Patient Data

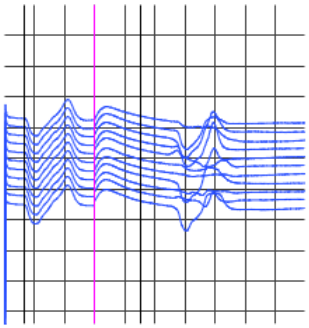
Patient ID: IBMS_D_Baseline#5
Name:
DOB:
Age:
Sex: Female
Height:

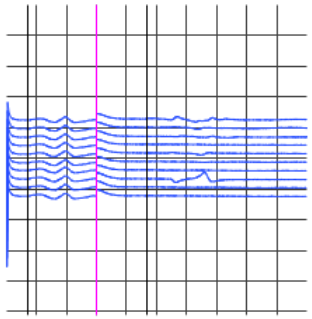
Date of Study: 05/04/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							22.7		
Median F-Response Right									
Wrist - APB							23.4		

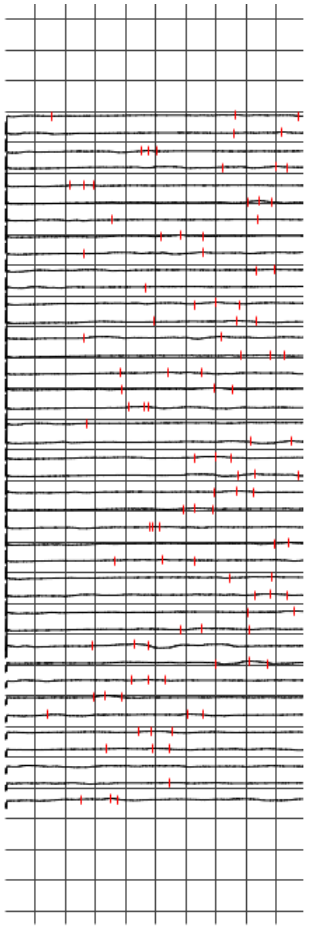
F-Wave

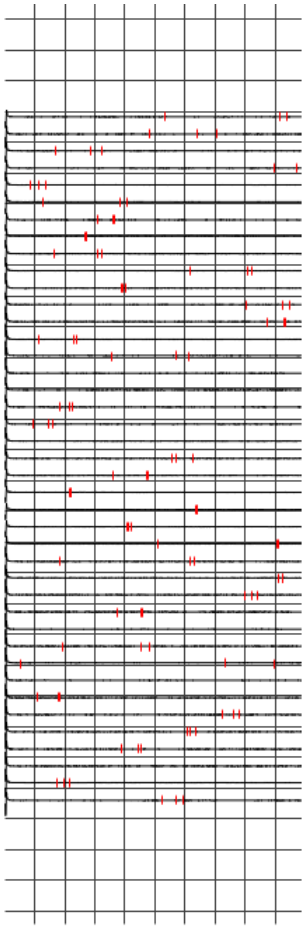




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	22.5	0.020	
		Right	15.9	0.019	
		Left-Right	6.6	0	





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Patient Data

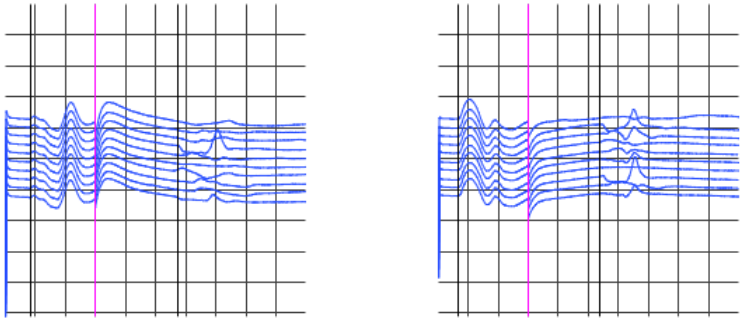
Patient ID: IBMS_D_Baseline#6
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 12/04/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

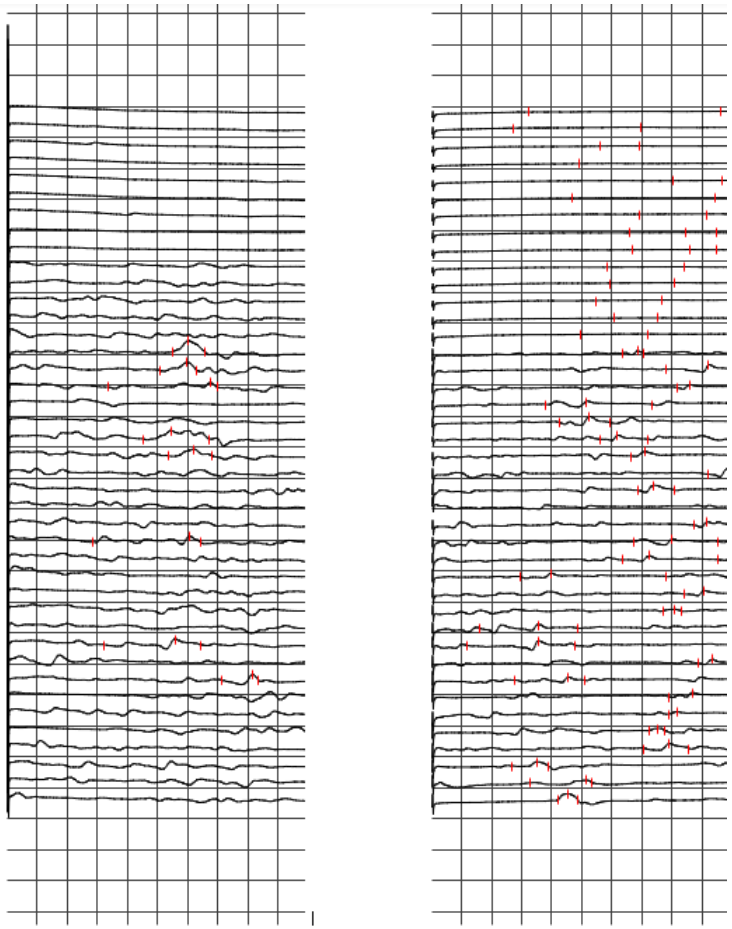
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							28.6		
Median F-Response Right									
Wrist - APB							26.9		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	--	--	
		Right	--	--	
		Left-Right	0	0	



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Patient Data

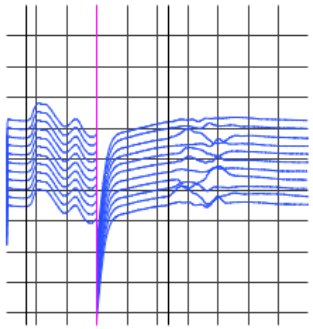
Patient ID: IBMS_D_Intervention#1
Name:
DOB:
Age:
Sex: Female
Height:

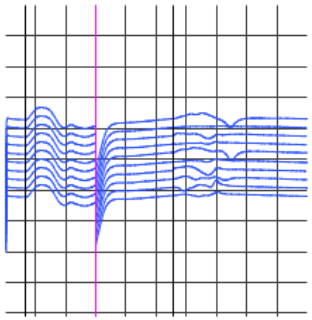
Date of Study: 10/05/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							26.9		
Median F-Response Right									
Wrist - APB							27.8		

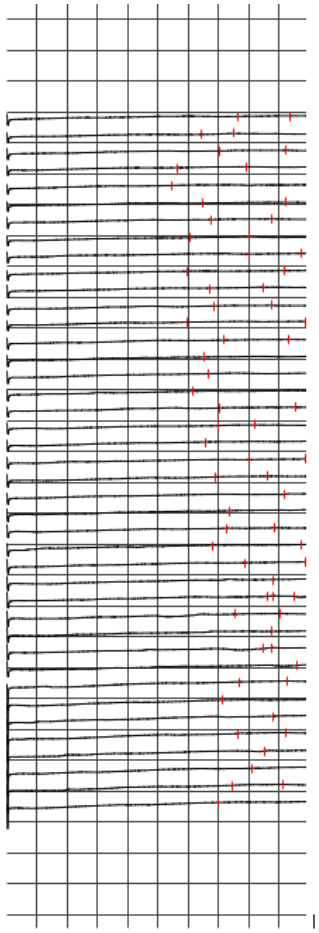
F-Wave

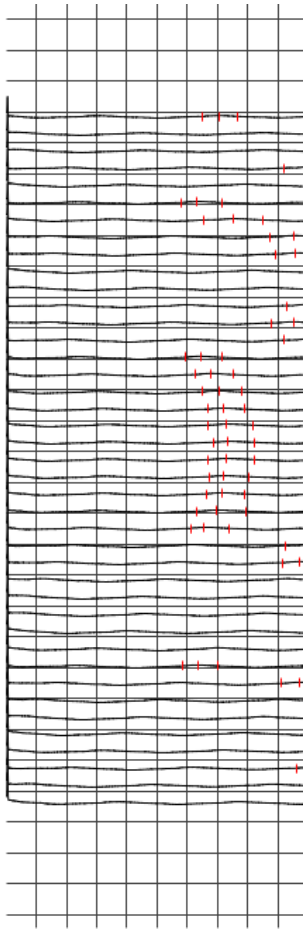




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	43.4	0.033	
		Right	24.5	0.022	
		Left-Right	18.9	0.011	





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Patient Data

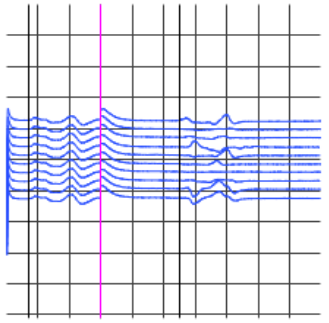
Patient ID: IBMS_D_Intervention#2
Name:
DOB:
Age:
Sex: Female
Height:

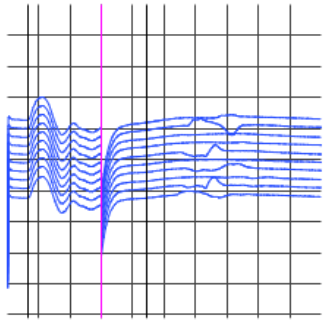
Date of Study: 24/05/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							27.6		
Median F-Response Right									
Wrist - APB							22.3		

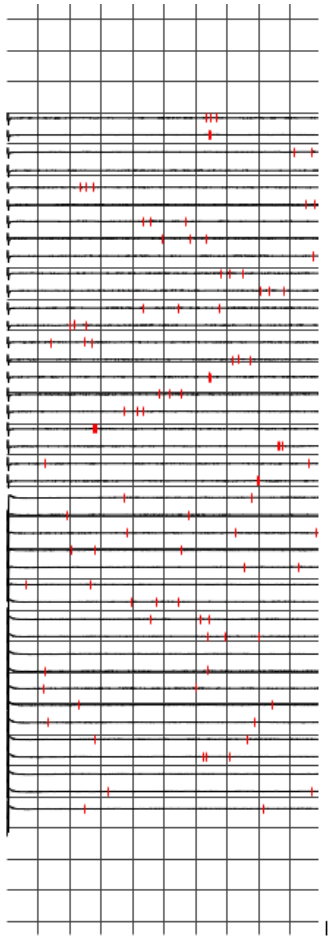
F-Wave

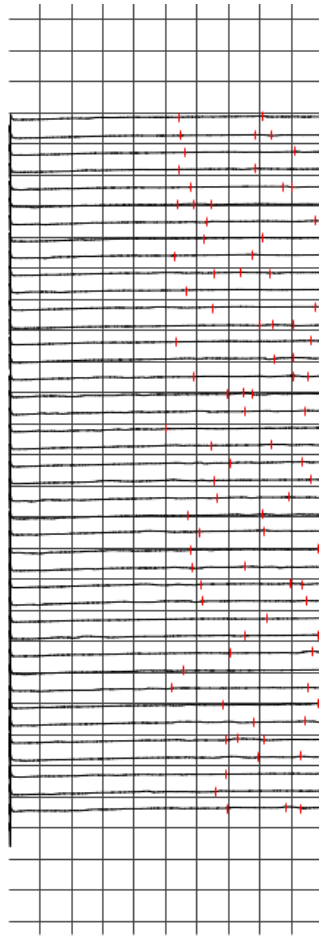




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	2.0	0.020	
		Right	26.7	0.083	
		Left-Right	24.7	0.063	





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Patient Data

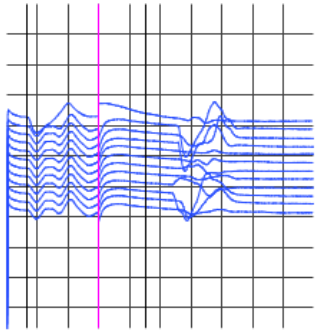
Patient ID: IBMS_D_Intervention#3
Name:
DOB:
Age:
Sex: Female
Height:

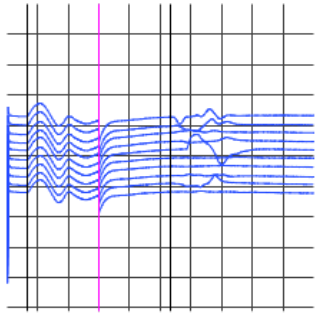
Date of Study: 07/06/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							22.6		
Median F-Response Right									
Wrist - APB							26.7		

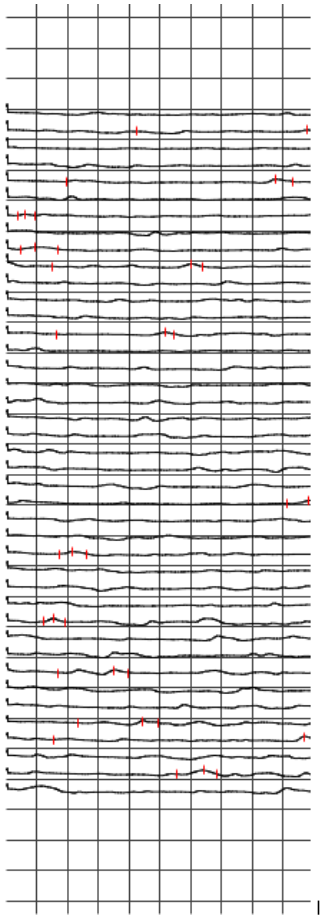
F-Wave

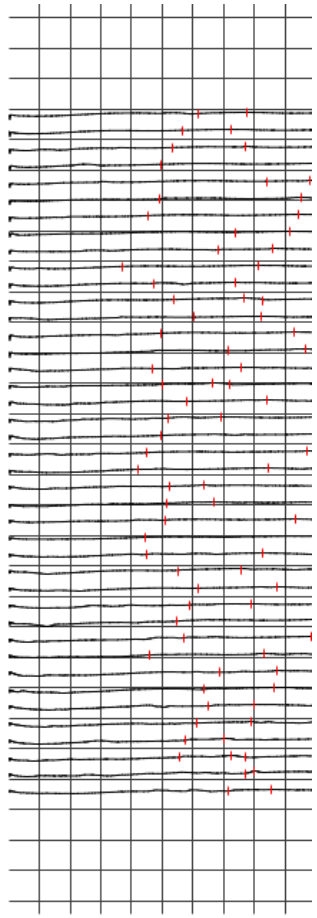




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	--	--	
		Right	--	--	
		Left-Right	0	0	





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Patient Data

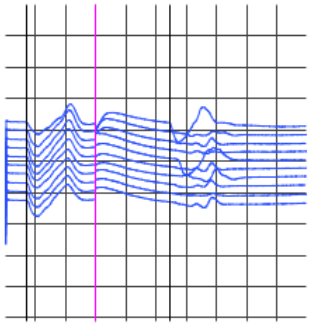
Patient ID: IBMS_D_Intervention#4
Name:
DOB:
Age:
Sex: Female
Height:

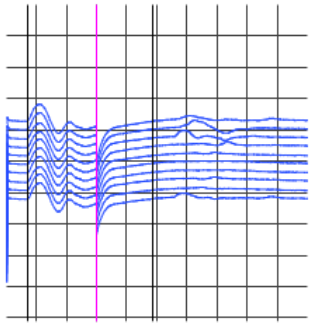
Date of Study: 21/06/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							27.3		
Median F-Response Right									
Wrist - APB							24.3		

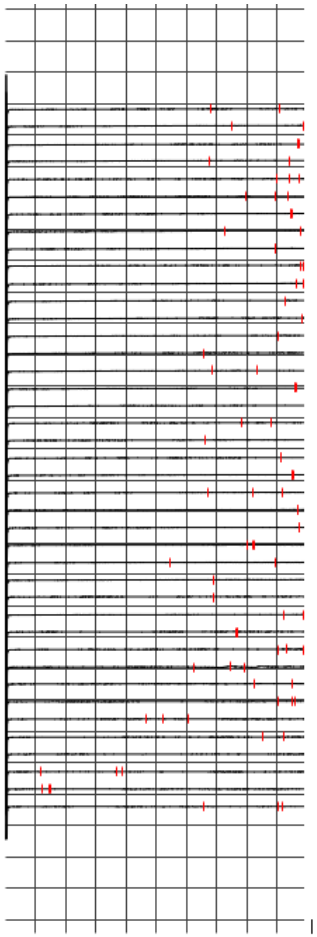
F-Wave

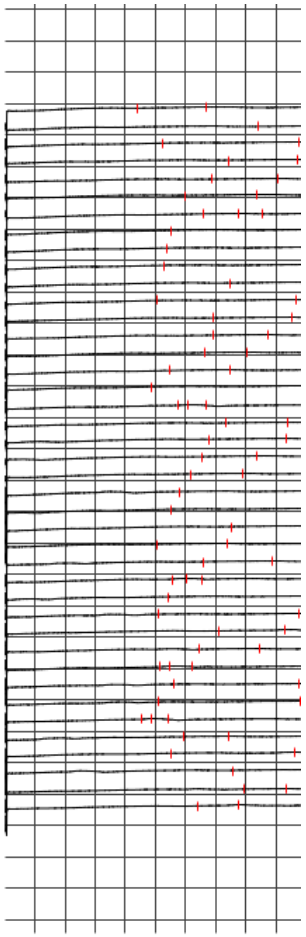




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	3.6	0.32	
		Left-Right	3.6	0.32	





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Patient Data

Patient ID: IBMS_D_Intervention#5
Name:
DOB:
Age:
Sex: Female
Height:

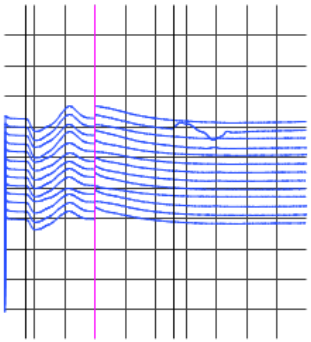
Date of Study: 05/07/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

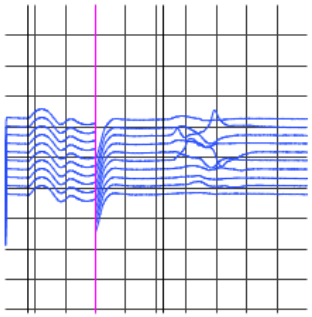
Nerve Conduction Studies - Motor

Motor NCS

Nerve	Lat (ms)	Lat ref limit	Ampl (mV)	Ampl ref limit	CV (m/s)	CV ref limit	F Lat (ms)	Distance (mm)	Limb Temp (°C)
Median F-Response Left									
Wrist - APB							27.9		
Median F-Response Right									
Wrist - APB							26.1		

F-Wave

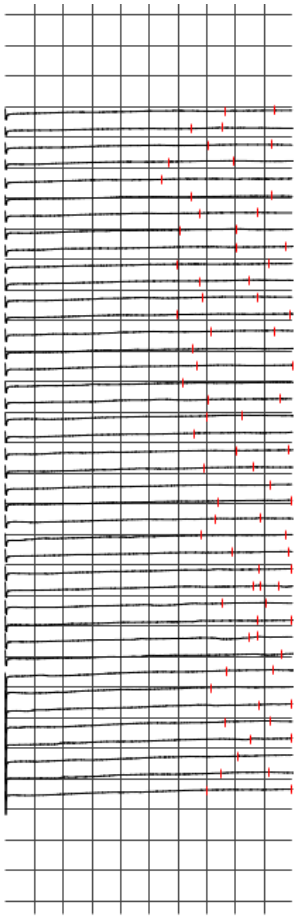


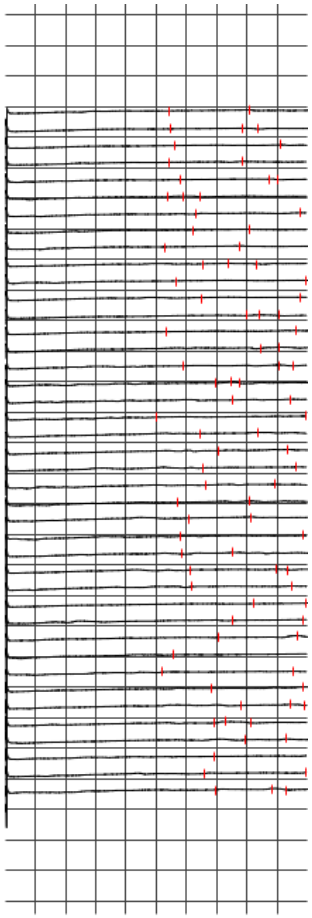


MEP Cortical/F

MEP

Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	--	--	
		Right	--	--	
		Left-Right	0	0	





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Patient Data

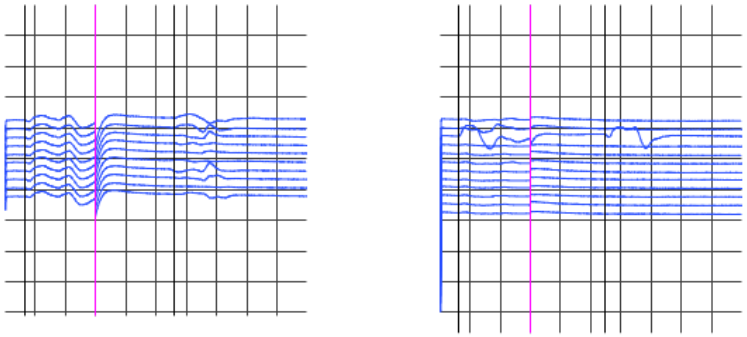
Patient ID: IBMS_D_Follow-up#1
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 02/08/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

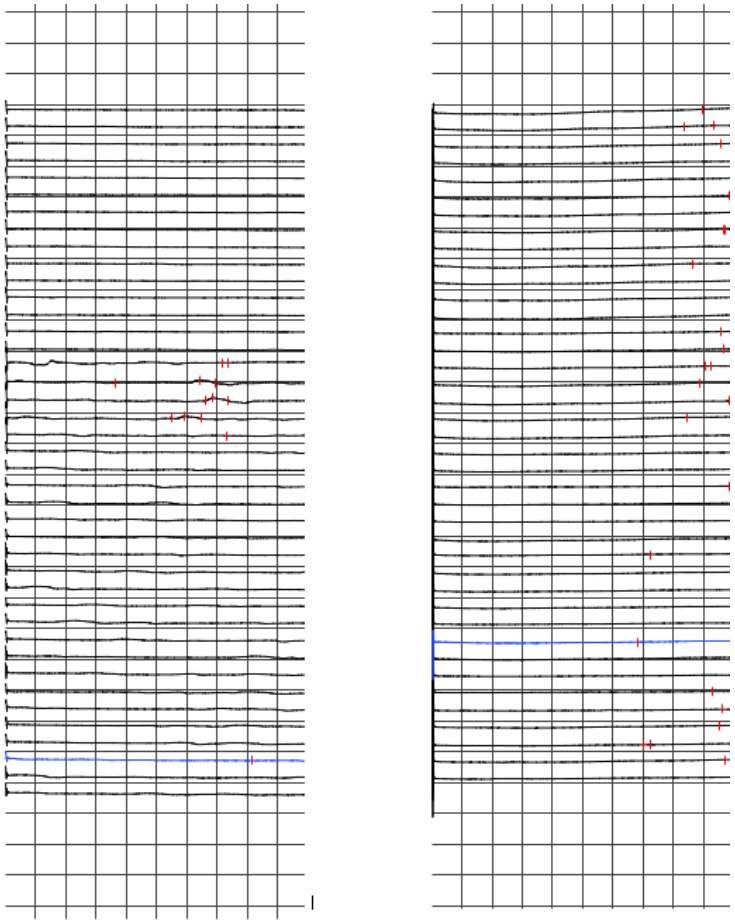
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							27.9		
Median F-Response Right									
Wrist - APB							27.4		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	OCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	40.8	0.052	
		Right	34.1	0.093	
		Left-Right	6.7	0.041	



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Patient Data

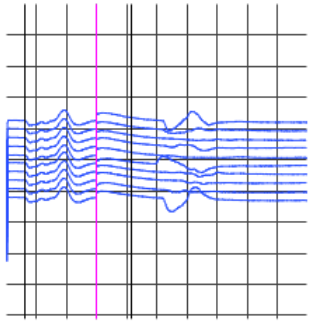
Patient ID: IBMS_D_Follow-up#2
Name:
DOB:
Age:
Sex: Female
Height:

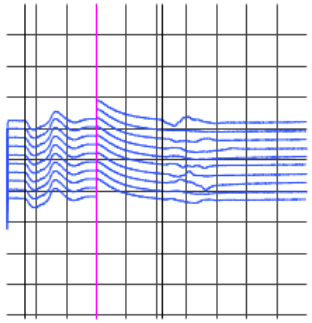
Date of Study: 30/08/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							20.8		
Median F-Response Right									
Wrist - APB							26.0		

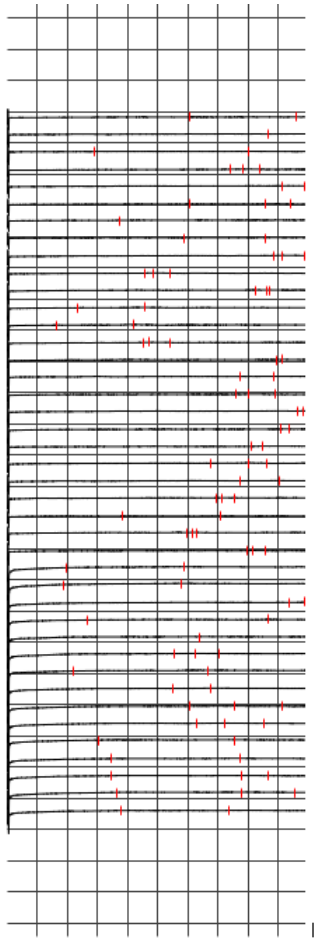
F-Wave

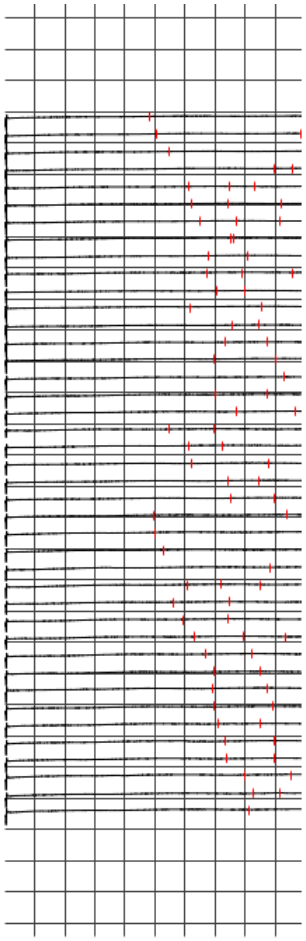




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	31.6	0.013	
		Right	--	--	
		Left-Right	31.6	0.013	





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Patient Data

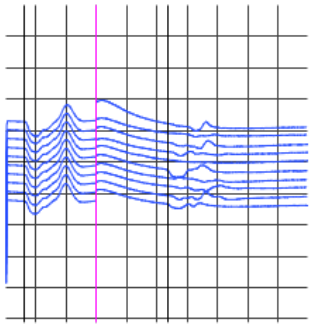
Patient ID: IBMS_D_Follow-up#3
Name:
DOB:
Age:
Sex: Female
Height:

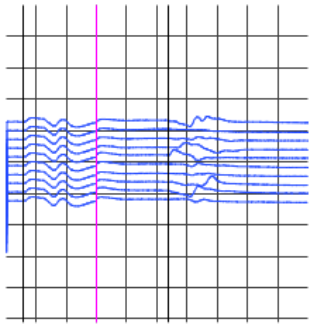
Date of Study: 27/09/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							26.8		
Median F-Response Right									
Wrist - APB							27.0		

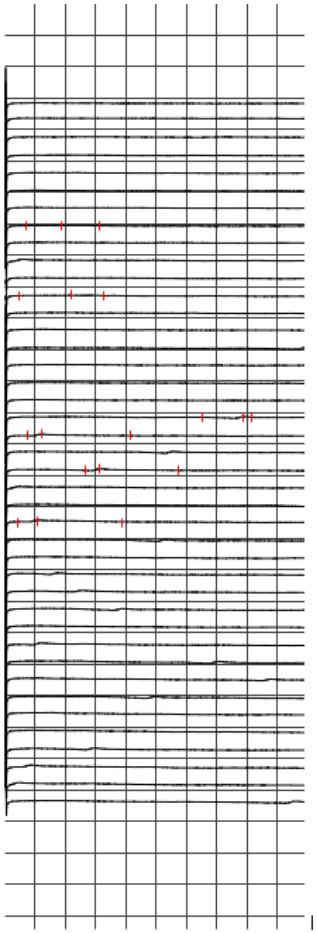
F-Wave

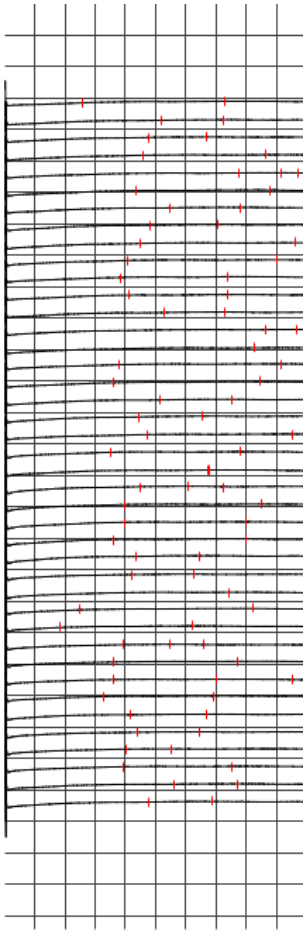




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	41.9	0.032	
		Right	--	--	
		Left-Right	41.9	0.032	





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Patient Data

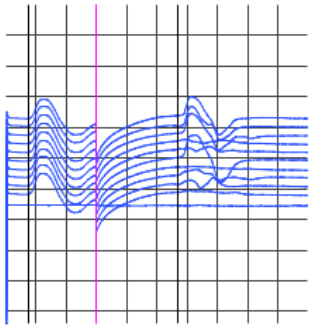
Patient ID: IBMS_E_Baseline#1
Name:
DOB:
Age:
Sex: Female
Height:

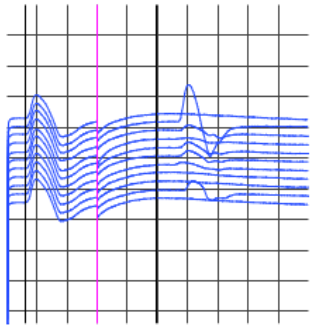
Date of Study: 10/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							28.4		
Median F-Response Right									
Wrist - APB							24.8		

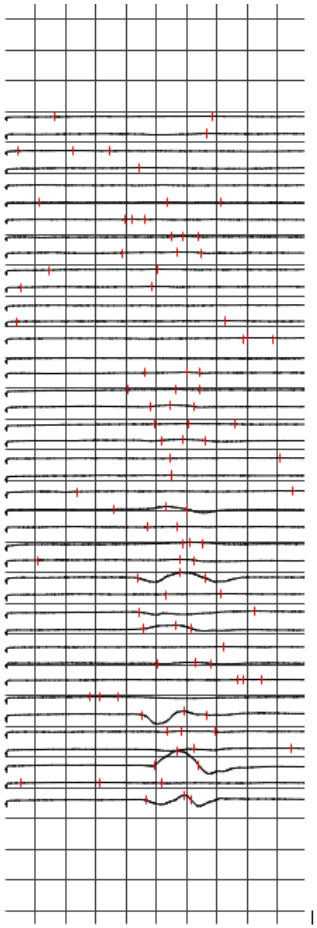
F-Wave

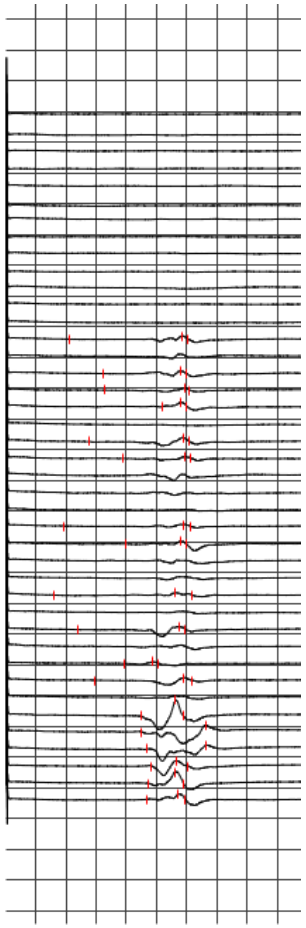




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	24.1	0.057	
		Right	--	--	
		Left-Right	24.1	0.057	





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Patient Data

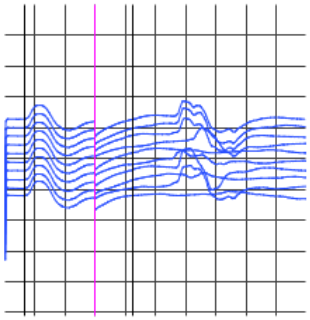
Patient ID: IBMS_E_Baseline#2
Name:
DOB:
Age:
Sex: Female
Height:

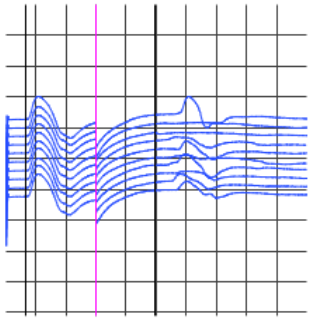
Date of Study: 17/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							21.3		
Median F-Response Right									
Wrist - APB							24.8		

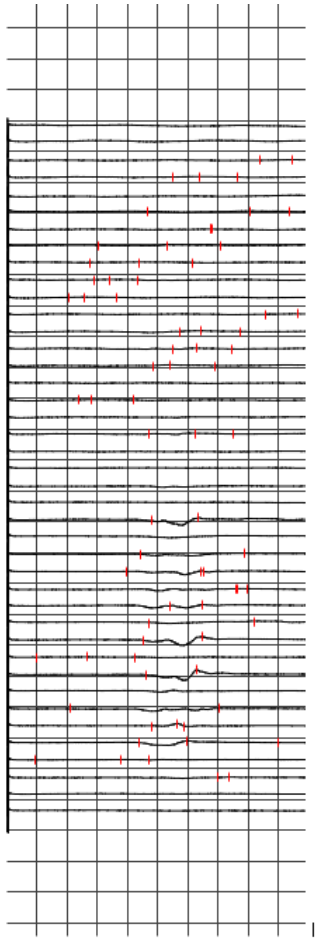
F-Wave

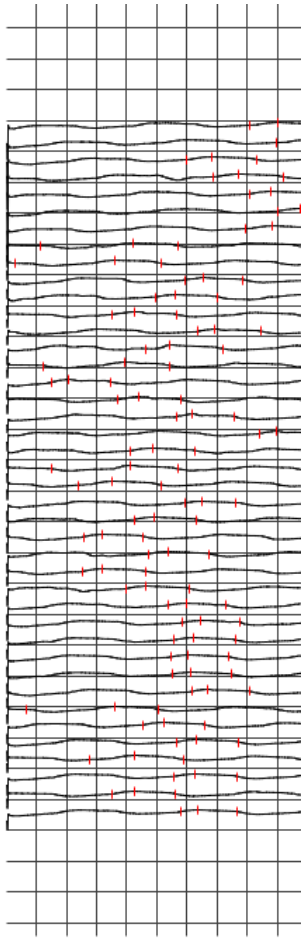




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	10.1	0.089	
		Left-Right	10.1	0.089	





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Patient Data

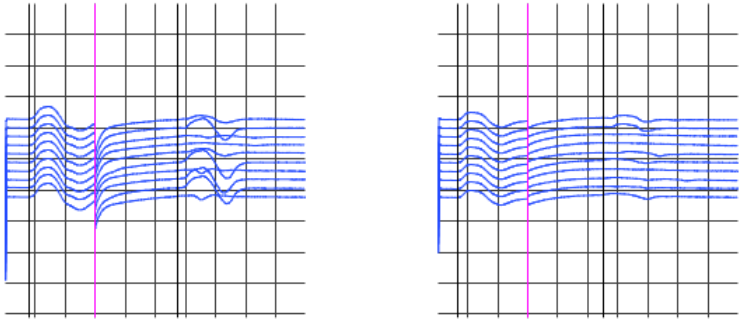
Patient ID: IBMS_E_Baseline#3
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 24/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

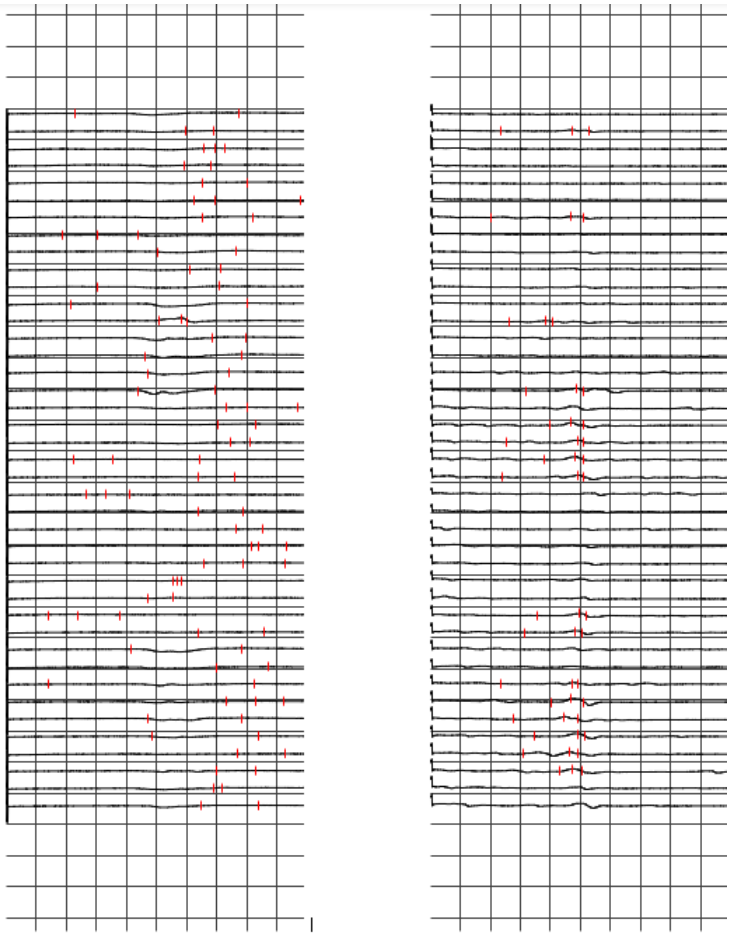
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							28.7		
Median F-Response Right									
Wrist - APB							27.7		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	24.4	0.40	
		Right	--	--	
		Left-Right	24.4	0.40	



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Patient Data

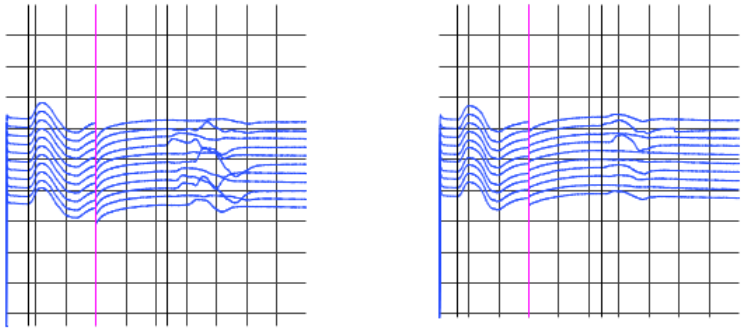
Patient ID: IBMS_E_Baseline#4
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 31/03/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

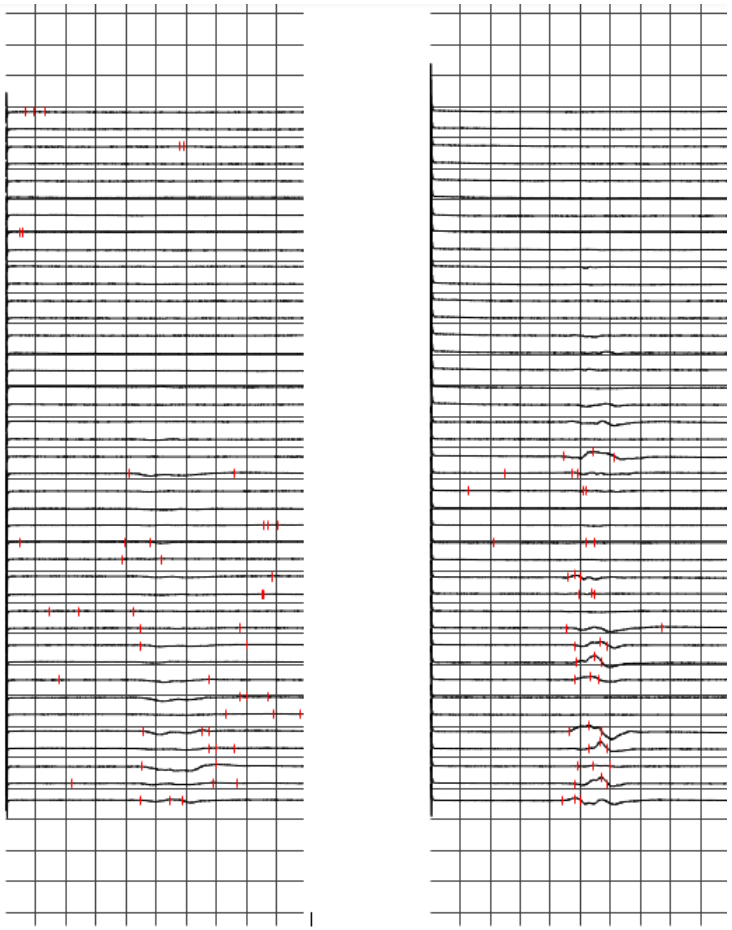
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							26.8		
Median F-Response Right									
Wrist - APB							27.0		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	31.1	0.060	
		Right	24.1	0.18	
		Left-Right	7.0	0.12	



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Patient Data

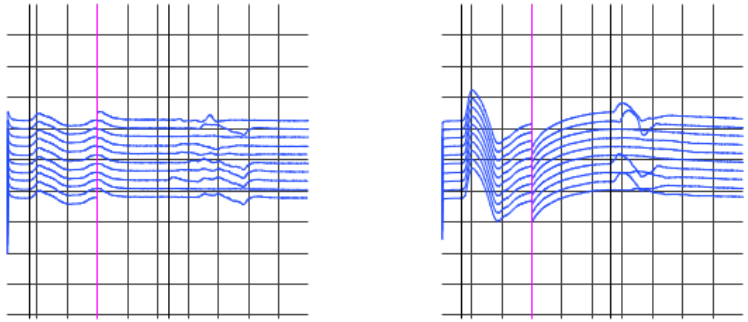
Patient ID: IBMS_E_Baseline#5
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 5/04/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

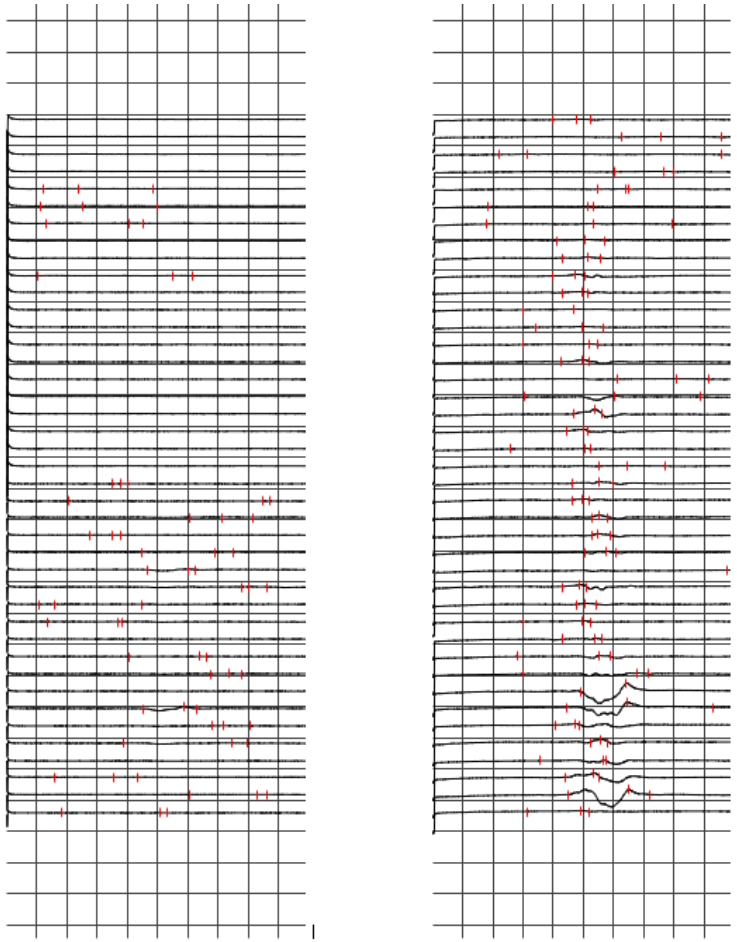
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							26.8		
Median F-Response Right									
Wrist - APB							28.0		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	23.1	0.10	
		Right	--	--	
		Left-Right	23.1	0.10	



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Patient Data

Patient ID: IBMS_E_Baseline#6
Name:
DOB:
Age:
Sex: Female
Height:

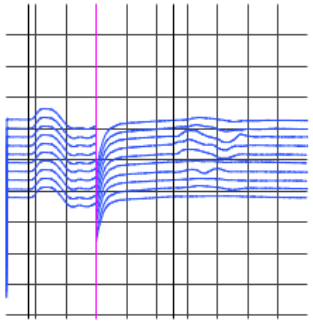
Date of Study: 12/04/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

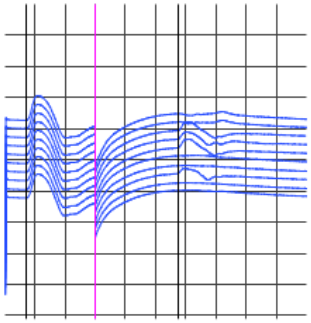
Nerve Conduction Studies - Motor

Motor NCS

Nerve	Lat (ms)	Lat ref limit	Ampl (mV)	Ampl ref limit	CV (m/s)	CV ref limit	F Lat (ms)	Distance (mm)	Limb Temp (°C)
Median F-Response Left									
Wrist - APB							27.8		
Median F-Response Right									
Wrist - APB							28.7		

F-Wave

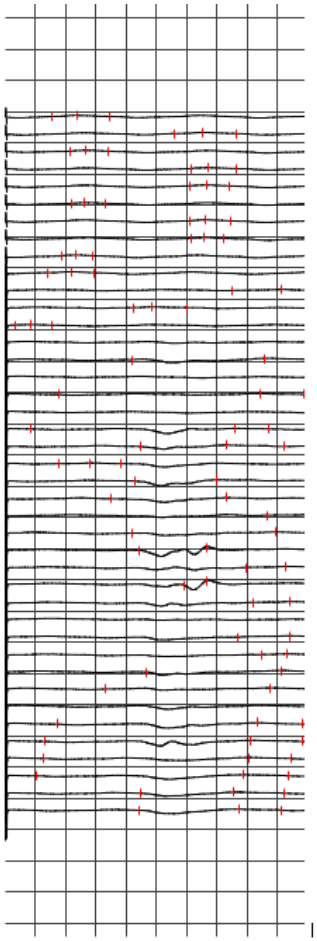


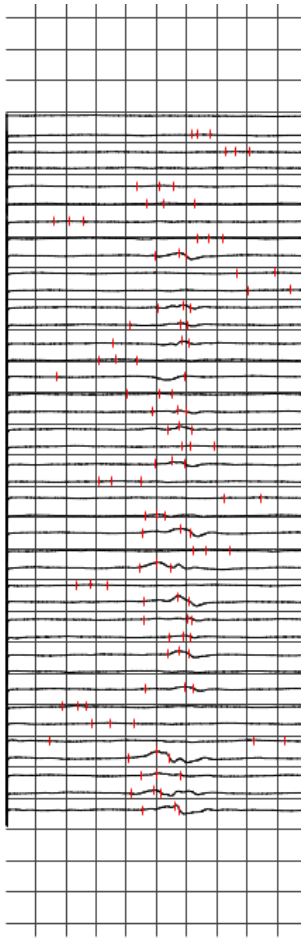


MEP Cortical/F

MEP

Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	48.6	0	
		Right	--	--	
		Left-Right	48.6	0	





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Patient Data

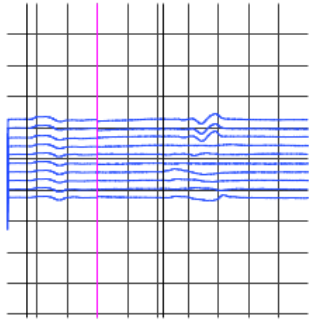
Patient ID: IBMS_E_Intervention#1
Name:
DOB:
Age:
Sex: Female
Height:

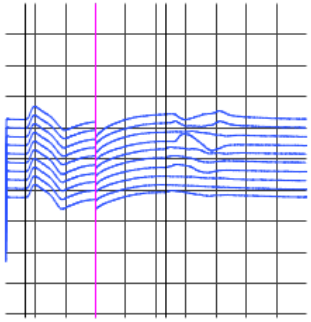
Date of Study: 17/05/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							26.0		
Median F-Response Right									
Wrist - APB							26.6		

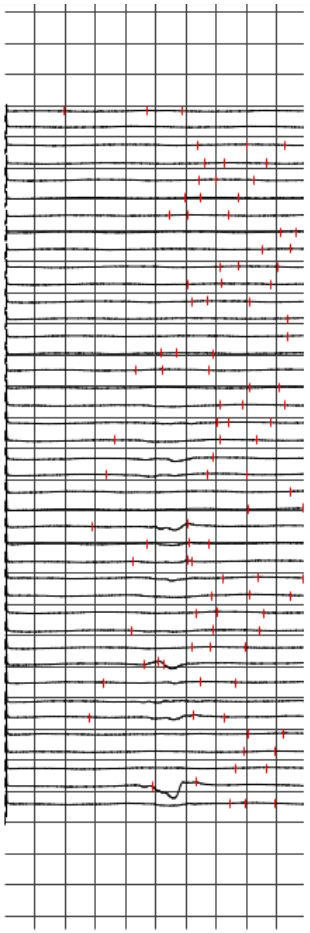
F-Wave

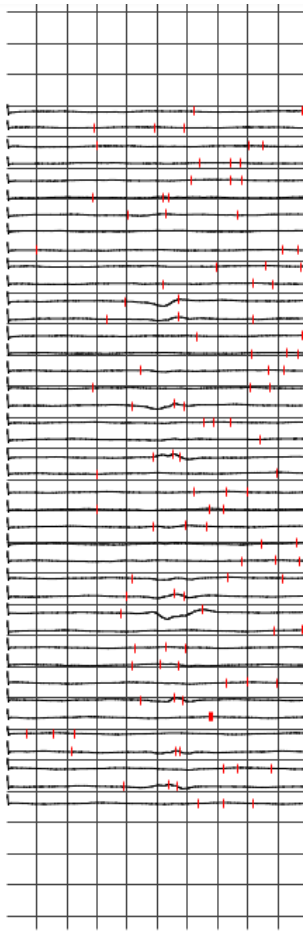




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat ms	Amp mV	CCT ms
Ulnar	Cortex - APB	Left	6.1	0.065	
		Right	--	--	
		Left-Right	6.1	0.065	





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Patient Data

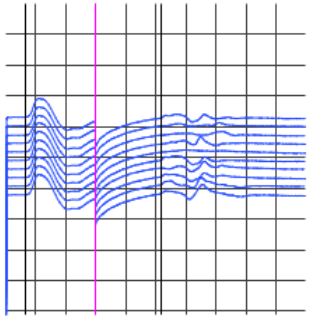
Patient ID: IBMS_E_Intervention#2
Name:
DOB:
Age:
Sex: Female
Height:

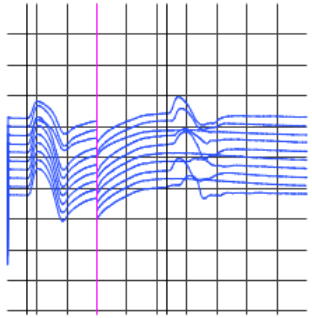
Date of Study: 31/05/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							25.9		
Median F-Response Right									
Wrist - APB							26.7		

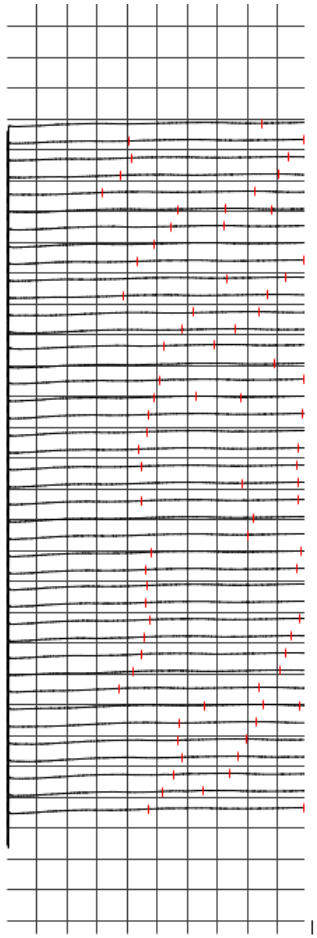
F-Wave

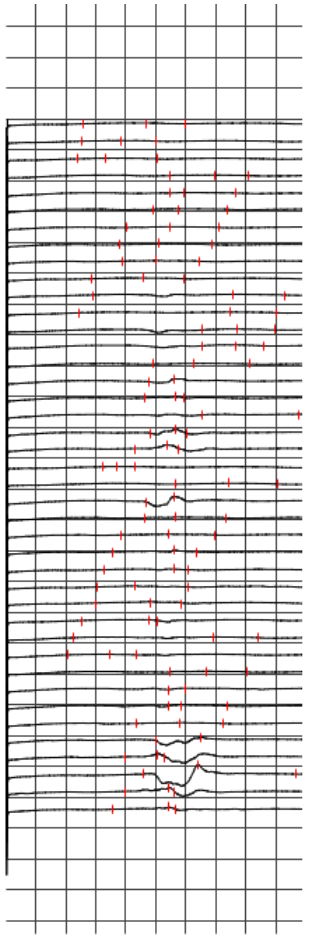




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	45.3	0	
		Right	22.1	0.74	
		Left-Right	23.2	0.74	





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Patient Data

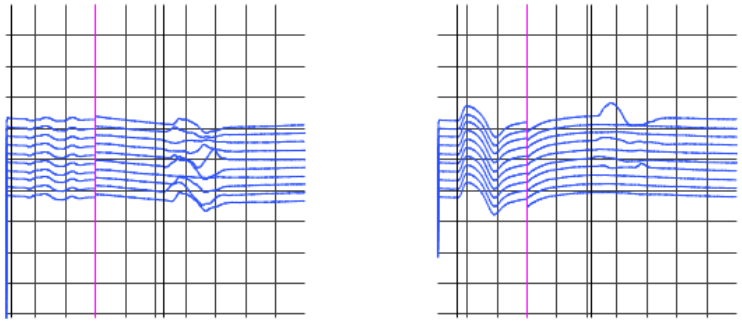
Patient ID: IBMS_E_Intervention#3
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 14/06/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

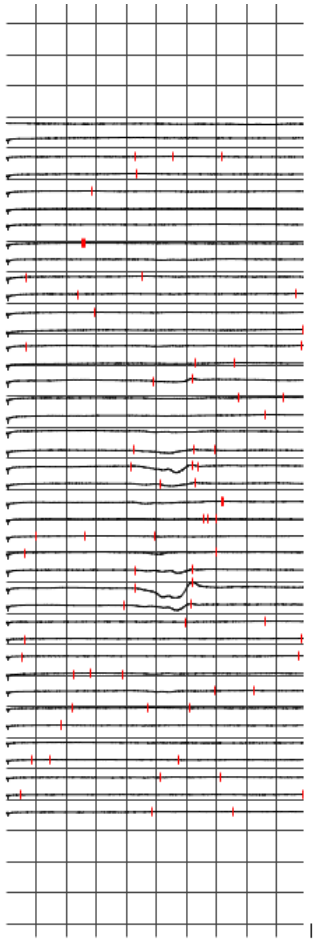
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							26.4		
Median F-Response Right									
Wrist - APB							25.7		

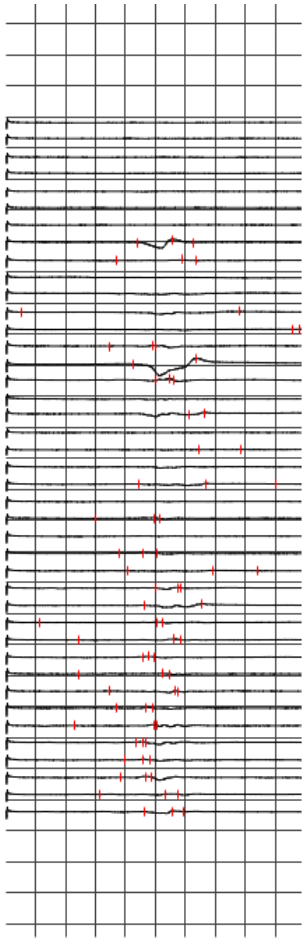
F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	45.1	0	
		Right	2.5	0.012	
		Left-Right	42.6	0.012	





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Patient Data

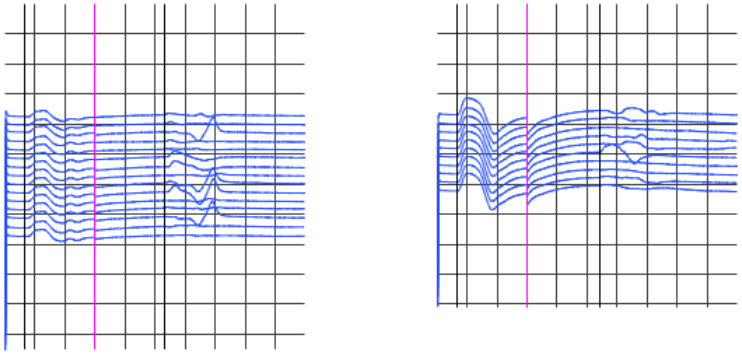
Patient ID: IBMS_E_Intervention#4
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 30/06/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

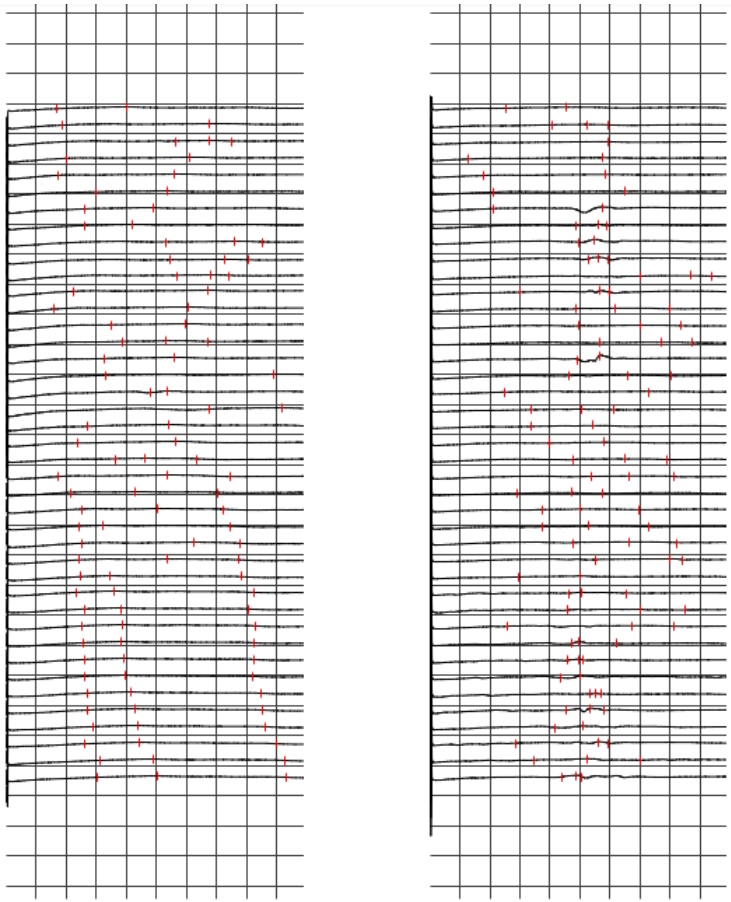
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							26.6		
Median F-Response Right									
Wrist - APB							27.1		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	29.1	0	
		Right	36.1	0.032	
		Left-Right	7.0	0.032	



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Patient Data

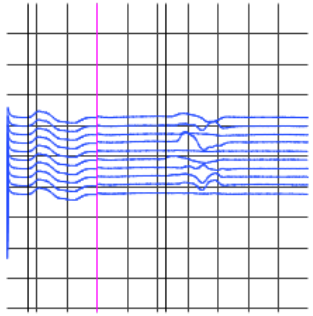
Patient ID: IBMS_E_Intervention#5
Name:
DOB:
Age:
Sex: Female
Height:

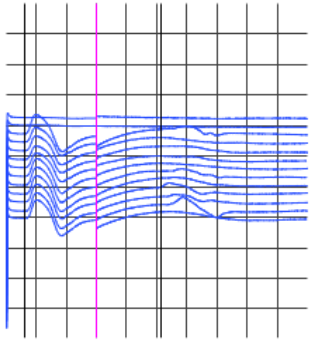
Date of Study: 14/07/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							26.3		
Median F-Response Right									
Wrist - APB							25.7		

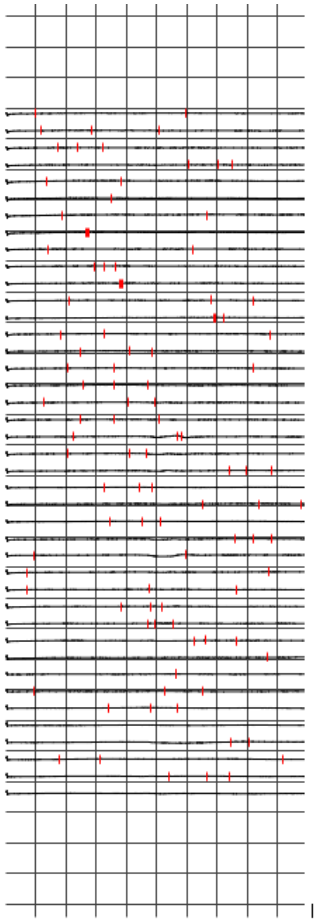
F-Wave

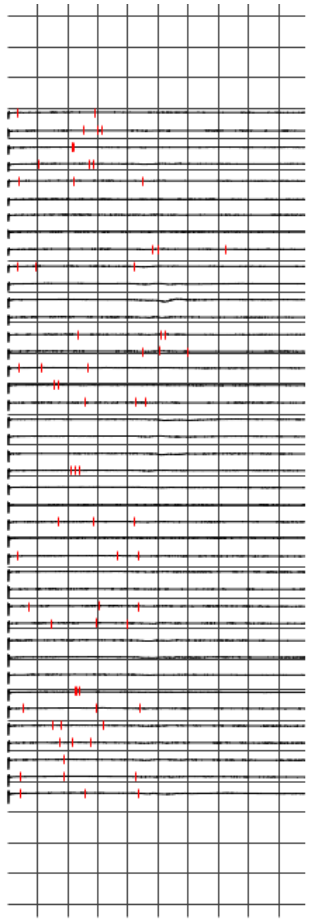




MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	CCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	--	--	
		Right	23.8	0.058	
		Left-Right	23.8	0.058	





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Patient Data

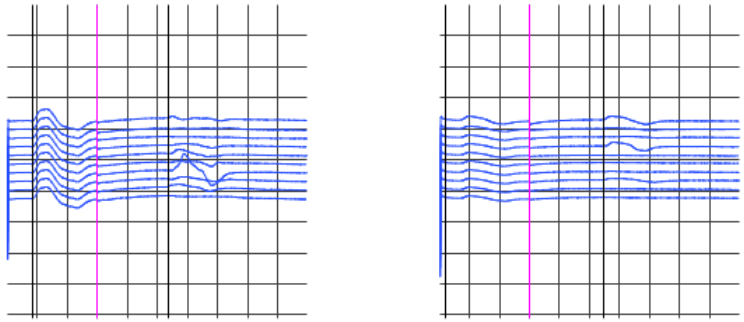
Patient ID: IBMS_E_Follow-up#1
Name:
DOB:
Age:
Sex: Female
Height:

Date of Study: 09/08/2023
Referring Physician:
Testing Physician:
Neurophysiologist:
History:

Nerve Conduction Studies - Motor

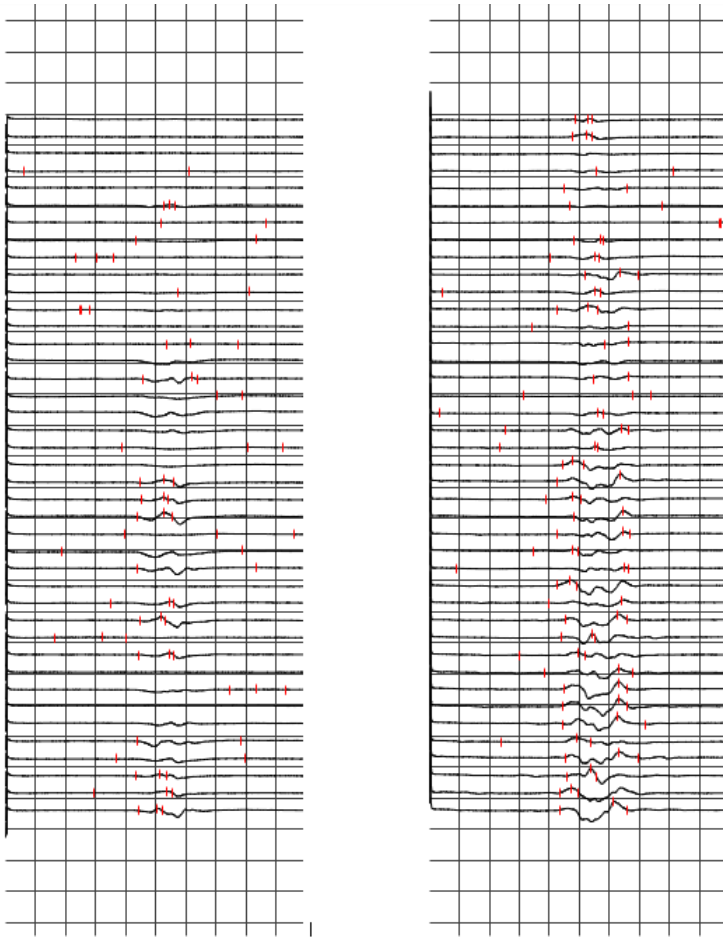
Motor NCS									
Nerve	Lat		Ampl		CV		F Lat	Distance	Limb Temp
	(ms)	ref limit	(mV)	ref limit	(m/s)	ref limit	(ms)	(mm)	(°C)
Median F-Response Left									
Wrist - APB							26.7		
Median F-Response Right									
Wrist - APB							27.4		

F-Wave



MEP Cortical/F

MEP					
Nerve	Site	Side	Lat	Amp	OCT
			ms	mV	ms
Ulnar	Cortex - APB	Left	23.7	0.18	
		Right	15.8	0.033	
		Left-Right	7.9	0.15	



APPENDIX X

In-phase bilateral upper limb exercises in pwPMS: SPIRIT Checklist Recommended items to address in a clinical trial protocol and related documents.

Section/item	ItemNo	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym The effects of in-phase bilateral exercise on cognitive and motor outcome measures in patients with Progressive Multiple Sclerosis, a randomized control trial. NCT06436131
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry ClinicalTrials.gov NCT06436131
	2b	All items from the World Health Organization Trial Registration Data Set - Primary registry and trial identifying number: ClinicalTrials.gov NCT06436131 - Date of registration in primary registry: 9 June, 2024

- Secondary Identifying numbers: N/A

- Source(s) of monetary or material support: Cyprus University of Technology

- Primary sponsor: Cyprus University of Technology

- Secondary sponsor(s): The Cyprus Institute of Neurology and Genetics, The Cyprus Foundation for Muscular Dystrophy Research

-Contact for public queries: DS; sokratous.physio@gmail.com; Cyprus University of Technology; Vragadinou 15, Limassol, 3041; 00357 25002294

- Contact for Scientific Queries: DS, NK, Cyprus University of Technology.

- Public title: The Effects of In-phase Bilateral Exercise in People with Progressive Multiple Sclerosis.

- Scientific title: The effects of in-phase bilateral exercise on cognitive and motor outcome measures in patients with progressive Multiple Sclerosis, a randomized control trial.

- Countries of recruitment: Cyprus

- Health condition(s) or problem(s) studied: exercise, clinical condition, cognitive functions and motor skills

- Intervention(s): In-phase bilateral exercise of the upper limbs in people with progressive multiple sclerosis.

- Key Inclusion and Exclusion Criteria:

Ages eligible for study: 30-70 years; Sexes eligible for study: both

Inclusion criteria: Patients with progressive multiple sclerosis (Primary and Secondary Multiple Sclerosis), Expanded Disability Status Scale score between three and six, Mini Mental State Examination score between 20 and 30 (mild to no cognitive impairment)

Exclusion criteria: Metal implants, history of cardiovascular or any disease affecting the central nervous system other than multiple sclerosis, pregnancy, epileptic seizures, Spasticity level on upper or lower limbs more than 1+ (slight increase in muscle tone) according to Modified Ashworth Scale.

- Study type:	Interventional
Estimated Enrollment :	20 participants
Allocation:	Randomized
Intervention Model:	Parallel Assignment
Intervention Model Description:	Subjects were randomized in two groups. Experimental group received an exercise program based on in-phase bilateral exercises, whereas the active control group receives conservative exercises. The duration of the study was 2 consecutive weeks for both groups.

- Masking:	Triple (Participant, Care Provider Outcomes Assessor)
Masking Description:	It was a double-blind study in which neither the participants nor the assessor and the trainer knew who's been assigned to either group.
Primary Purpose:	Basic Science
- Date of first enrolment:	2/9/2024
- Sample size:	20
- Recruitment status of this trial:	Participants were recruited and enrolled.
- Primary Outcome(s):	Symbol Digit Modalities Test [Time Frame: Through study completion, Baseline (3 weeks) until the end of the Intervention (12 weeks)].
- Key secondary outcomes:	Medical Outcomes Study Short Form 36 Modified Fatigue Impact Scale Purdue Pegboard Test Timed 25-Foot Walk Six Spot Step Test

			[Time Frame: Through study completion, Baseline (3 weeks) until the end of the Intervention (12 weeks)].
		- Ethics Review:	Status: Registration No. ΕΕΒΚ ΕΠ 2022 32 Date of approval: 10/6/2022 Name and contact details: Cyprus National Bioethics Committee; 22 La (00357) 22-809038 / 22-809039 / 22819101 cnbc@bioethics.gov.cy
		- Completion date:	December 2023 is the study completion date.
		- IPD sharing statement:	Undecided
Protocol version	3	Date and version identifier Issue date: 9 June, 2024 Authors: D.S., E.Z.P., K.M. and N.K.	
Funding	4	Sources and types of financial, material, and other support	

		Equipment, consumables and diagnostic devices have been provided by The Cyprus Institute of Neurology and Genetics.
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors

D.S. [Department of Rehabilitation Sciences, Faculty of Health Sciences, Cyprus University of Technology, Limassol, Cyprus;

Physiotherapy Unit, Neurology Clinics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus].

E.Z.P. [Neuroepidemiology Department, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus].

N.K. [Department of Rehabilitation Sciences, Faculty of Health Sciences, Cyprus University of Technology, Limassol, Cyprus].

K.M. [Biostatistics Unit, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus].

Author contribution

D.S., E.Z.P., and N.K., are responsible for the conception and the experimental design.

D.S., and K.M., are responsible to collect, analyse and interpret the data.

D.S., and N.K., are responsible to draft the manuscript.

N.K. and K.M., revised the manuscript critically for important intellectual content.

5b Name and contact information for the trial sponsor

Investigator Name: Mr. Dimitris Sokratous

Investigator Official Title: MSc

Investigator Affiliation: Cyprus University of Technology, Department of Rehabilitation Sciences, Cyprus University of Technology. Vragadinou 15, Limassol, 3041, 00357 25002294

5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities

N/A

5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)

Principal investigator (PI)

Design and revision of the protocol

Organising steering committee meetings

Managing CTO [clinical trials office]

Publication of study reports

Members of TMC [Trial Management Committee]

Steering committee

(PI, research consultant, senior neurologist, senior physiotherapist)

Agreement of final protocol

All lead investigators will be steering committee members.

Recruitment of patients and liaising with principle [sic] investigator

Reviewing progress of study and if necessary agreeing changes to the protocol and/or facilitate the smooth running of the study.

Trial management committee

(PI, research physician, senior physiotherapist, administrator)

Study planning

Organisation of steering committee meetings

Responsible for trial master file

Advice for lead investigators

Data verification

Randomisation

Data manager committee

(senior physiotherapist, biostastician)

Maintenance of trial IT system and data entry

Data verification

Analysis plan

Data collection and completion

Lead investigators

Senior neurologist and senior physiotherapist are responsible for identification, recruitment.

Lead investigators will be steering committee members.

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention

Introduction: The term progressive Multiple Sclerosis (MS) includes both Secondary Progressive MS and Primary Progressive MS. Patients with progressive MS, except from the physical impairment, often have cognitive dysfunction, which negatively affects their quality of life. Information processing speed is the most common cognitive deficit in both Primary Progressive MS and Secondary Progressive MS .

Mechanism: Evidence from previous studies reported that in-phase bilateral movements need less attentional load and less motor control than that of unilateral or the other types of bilateral coordination, resulting to easily and efficiently performance of the specific type of movement (i.e., in-phase bilateral). Given that patients with PMS are characterized by a decline in information processing speed which ultimately affects manual dexterity, a reasonable question arises as to whether in-phase bilateral upper limbs exercises will improve information processing speed and thus, improve manual dexterity in the specific clinical cohort.

Existing knowledge: Results from previous studies in healthy population, indicated a linear relationship between cognitive function and upper limb performance in both healthy and people with Multiple Sclerosis. Furthermore, previous studies reported that bimanual hand coordination is connected with a symmetric facilitation of neural activity arbitrated by both increased intrahemispheric connectivity and greater

transcallosal coupling of SMA and M1. In addition to this relationship, Grefkes et al., (2008) indicated the efficacy of in-phase bilateral upper limbs movements on the interhemispheric connectivity between SMA and M1 for both brain hemispheres.

Need for a trial: In light of evidence that information processing speed is the most common cognitive deficit in both Primary Progressive MS and Secondary Progressive MS and given that there is a close relationship between cognitive function and upper limb performance, these findings raise the question about the effects of bilateral exercises on cognitive processing. Such effects would provide strong evidence about whether exercise, in particular in-phase bilateral exercise, can influence enhance information processing speed in people with progressive Multiple Sclerosis.

6b Explanation for choice of comparators

According to the MS guidelines, various types of exercises are recommended for improvement of cognitive and motor symptoms. Therefore, a within, as well as a between groups analysis and comparison can be used to identify the possible effects. Changes in clinical symptoms of the participants of the experimental group, compared to the active control group, during the three experimental phases (i.e., baseline, intervention, follow-up), will encourage the effectiveness of the proposed intervention.

Objectives	7	Specific objectives or hypotheses **7.1. Research hypothesis** In-phase bilateral exercises of the upper limbs, improve cognitive processing and clinical condition of people with Progressive Multiple Sclerosis. **7.2. Primary objectives** To determine if the exercises which include in-phase bilateral movement for the upper limbs can improve cognitive processing of people with Progressive Multiple Sclerosis. **7.3. Secondary objectives** To determine if the exercises which include in-phase bilateral movement for the upper limbs can improve the clinical condition of people with Progressive Multiple Sclerosis.
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) The study follows a double blind parallel group design. The allocation of participants into the two groups was conducted by an independent researcher, who organized the division to ensure the homogeneity of the groups, based on the individual EDSS score, age, gender and hand dominancy. These four parameters were chosen as the criteria for the group allocation, due to their impact in exercise performance and due to the direct

relationship with the study's outcome measures. As far as we know, increase disability level (i.e., EDSS) and the age negatively impact exercise performance. Also, gender is an important parameter affecting exercise performance, with women generally exhibiting lower performance levels than men. Lastly, because our research hypothesis it is based on the bilateral hand dexterity, the criterion of hand dominancy could have an impact in the study's results.

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained The intervention of the experimental group was conducted in a dedicated fitness room provided by the Cyprus Sport Association specifically for the purposes of this study. The intervention of the active control group and all the assessments for both study groups were conducted in the physiotherapy unit of The Cyprus Institute of Neurology and Genetics (CING).
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)

All participants were recruited and evaluated by the neurologist of CING. Moreover, all participants read and sign a written informed consent while all procedures were approved and conducted in accordance with the ethical guidelines of the Cyprus National Bioethics Committee before recruitment (see Appendix 1_Informed Consent Form).

10.1. Inclusion criteria

1) diagnosed with RRMS,

2) Expanded Disability Status Scale score between three and six,

3) aged between 30 and 70 years,

4) no relapse within 30 days,

5) Mini Mental State Examination score between 20 and 30 (no cognitive impairment).

10.2. Exclusion criteria

1) metal implants,

2) history of any disease affecting the central nervous system other than MS,

3) history of cardiovascular disease,

		4) mental disorders, 5) severe orthopaedic disorders, 6) pregnancy, 7) visual deficit, 8) hearing impairments, 9) epileptic seizures, 10) spasticity level on upper or lower limbs more than 1+ (slight increase in muscle tone) according to Modified Ashworth Scale.
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered According to the study design (i.e., multiple baseline design across subjects, the intervention was introduced systematically in one patient while baseline data collection continues in the others without any intervention. During the experimental procedure, all participants began the study with the baseline phase at the same time while the intervention phase was introduced staggered across patients and time. Therefore, five people with RRMS were allocated randomly. The intervention protocol lasted for 12 consecutive weeks (30-60 minutes /session x 3 sessions/week) and included in-phase bilateral movements of the upper limbs, adapted to different

sports activities and to functional training. Specifically, each session consisted of one to three sets, consisting of one minute continues repetitions of 9 different exercises targeting large muscle groups of the upper limbs (shoulder flexors, extensors, rotators, abductors and adductors, elbow flexors and extensors, hand and finger flexors and extensors). Additionally, three exercises targeted large lower limb muscle groups (hip flexors, extensors, abductors and adductors, knee and ankle flexors and extensors) to be performed in between the upper limbs exercises to allow relaxation of the upper limb muscles.

The specific exercises included sports activities of basic technical skills of basketball (e.g., different types of passing, catching and throwing the ball) and volleyball (e.g., different types of passing and receiving the ball), whereas the fitness exercises included the diagonal movements from proprioceptive neuromuscular facilitation technique. To maintain the interest of the participants, the exercise program was modified throughout the course of the 12-week intervention period via changing the level of difficulty. For example, elastic bands with different resistance levels were used. Every intervention session consisted of a five minutes' warm-up (i.e., whole body range of motion exercises), followed by the main sport activities and fitness exercise protocol as described above, and a cool down session for five minutes (i.e., passive stretching exercises of the muscle groups which are involved in the main part).

11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)

Fatigue

In order to maintain individual fatigue, each exercise protocol was adapted to the individual needs and with sufficient resting time, as well as with continues monitoring.

However, each participant had to complete at least 27 (75%) out of 36 sessions in order to be included in the data analysis.

11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)

Face-to-face adherence information sessions for all participants took part before starting the experimental procedures. These sessions included:

- The importance of following study guidelines and instructions about the type of exercises and the specific assessment procedures.

- Importance of calling nurses or/and doctors if experiencing any problems or symptoms possibly related to the study.

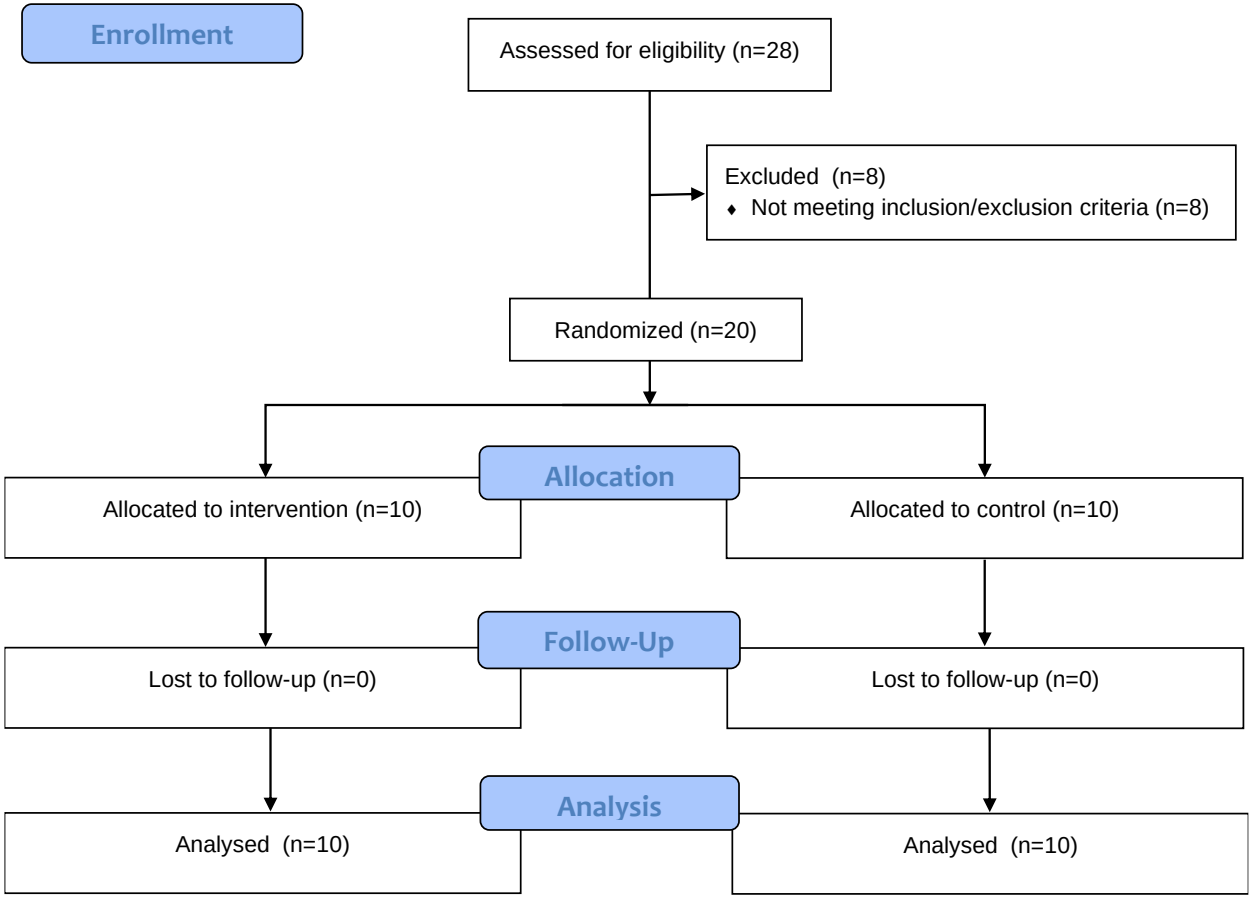
- There was a brief discussion of reasons for feeling any unexpected symptoms (e.g., pain, fatigue).

		Moreover, participants had the opportunity to ask questions from the initial session and reviewed as needed. **Adherence assessments** To enhance validity of data, an individual electronic data form was used to record all neurophysiological and clinical assessments, which was stored in a secure study computer
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial Participants were advised to continue their usual prescribed medication throughout the study duration, and they were advised to continue their usual daily routine avoiding receiving any other exercise program during the study.
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended **Primary Outcome Measures** We analysed data from the Symbol Digit Modality Test, to determine individual information processing speed and therefore to test the primary hypothesis.

Secondary Outcome Measures

We investigated the effects of the specific exercises protocol on various clinical outcomes (i.e., gait, balance, hand dexterity). The Purdue Pegboard Test, which is a standardized test of manual dexterity, the Timed 25-Foot Walk which is a quantitative assessment for mobility and lower limb function, the Six Spot Step Test is an assessment tool that evaluates a complex range of sensorimotor functions, part of which are lower limb strength, spasticity, coordination, as well as balance. Finally, we performed the Modified Fatigue Impact Scale which is a short questionnaire that requires the participants to describe the effects of fatigue during the past four weeks and the Medical Outcomes Study Questionnaire Short Form 36 Health Survey. This is a set of generic, coherent, and easily administered quality-of-life subjective questionnaire.

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. For the experimental procedures see also Appendix 3_ Figure 1.


Enrollment
Assessed for eligibility (n=28)
Excluded (n=8)
♦ Not meeting inclusion/exclusion criteria (n=8)
Randomized (n=20)
Allocation
Allocated to intervention (n=10)
Allocated to control (n=10)
Follow-Up
Lost to follow-up (n=0)
Lost to follow-up (n=0)
Analysis
Analysed (n=10)
Analysed (n=10)

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations This is a two-arm, double blind randomized control trial, which evaluated a 12-week exercise program to determine the effects of in-phase bilateral upper limbs exercise on information processing speed in people with Progressive Multiple Sclerosis. To achieve the study objectives, participants were randomly allocated to two independent groups: experimental and active control group.
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size One of the affiliated organizations is the Cyprus Institute of Neurology and Genetics, which is a medical and biomedical translation centre, promoting patient care, research and educational programs on neurological disease, including Multiple Sclerosis. In regards to participants recruitment an information letter was given to the outpatients of The Cyprus Institute of Neurology and Genetics between May 2023 and August 2023, to express their interest for participation in the current study. Therefore, patients will be recruited mainly from the senior neurologist of CING throughout the outpatient registry.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions The allocation of participants into the two groups was conducted by an independent researcher, who organized the division to ensure the homogeneity of the groups, based on the individual EDSS score, age, gender and hand dominancy.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned The randomization process for the participants' assignment was performed via Microsoft Excel 2021 software and based on the four parameters; EDSS score, age, gender and hand dominancy. Firstly, a list of participants was created by a unique identifier. Secondly, the independent researcher categorized all participants according to the individual EDSS score. Thirdly, the participants were manually

		placed alternately in the two groups, so that there were equal numbers of participants in each group based on the EDSS score. Fourthly, the participants in both groups were re-allocated based on the individual age, so that the mean age to be equal between the two study groups and at the same time to have equal mean EDSS score between the groups. The next step was again a re-allocation of the participants in both groups based on gender, so that the female to male ratio to be equal between the two study groups and at the same time have equal mean EDSS score and mean age between the groups. Finally, the participants in both groups were re-allocated based on the hand dominancy, so that the left to right handed ratio is equal between the two study groups and at the same time have equal mean EDSS score, mean age and equal female to male ratio between the groups.
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions All patients who fulfilled the inclusion criteria were randomized and then informed about the study procedures, so finally the consent for participation was given. Randomisation requested by the senior neurologist responsible for recruitment according inclusion/exclusion criteria from the Cyprus Institute of Neurology and Genetics. Then the list of participants' randomised order was sent to the main researcher (i.e., senior physiotherapist of the Cyprus Institute of Neurology and Genetics) who coordinated the study. The therapist gave the information about the exercise protocol and the related neurophysiological and clinical assessments.

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how This is a parallel group, double-blind study in which neither the participants nor the assessor, the trainer and data analyst knew who's been assigned to either group.
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial Since the current study was a double blind randomized control trial, none of the participants or the assessors could be unblinded. The only exception was in the case of adverse effects, therefore only the principal investigator could be unblinded.

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol

Primary Outcome Measures

Symbol Digit Modalities Test. It is a commonly used test in people with Multiple Sclerosis, which measures processing speed as well as motor speed. The investigators employed the oral form of the test, in which participants were provided with the test sheet with nine symbols, each paired with a number on top of the page, defined as the "key". For example, the symbol "O" is matched with the number "6", so the correct response would be "six". The rest of the page consists of a randomized, sequential variety of these symbols. Participants are asked to verbally respond with the number that corresponds with each symbol. During the test, the participant is given two minutes to orally match symbols with digits as quickly as possible. The score is obtained by subtracting the number of errors from the number of items completed. To account for practice effects, the investigators created six different tests, as many as our assessment points, in which the order of the symbols and the numbers of the "key" were rearranged.

Secondary Outcome Measures

1) Medical Outcomes Study Short Form 36; It is a set of generic, coherent, and easily administered quality-of-life measures, completed by the participants. There are 11 questions in the specific questionnaire administered by an assessor, with 36 items in total, which cover eight domains scaled from 0 to 100, with higher values indicating better health status. The eight domains include: general health, vitality, physical function, role physical, bodily pain, role emotional, social functioning and mental health. It takes between 5 and 10 min to complete it. 2) Modified Fatigue Impact Scale; It is a short questionnaire which requires the participants to

describe the effects of fatigue during the past four weeks. The Modified Fatigue Impact Scale consists of 21 questions which are subjectively rated from "0" (low rate) to "4" (high rate) and it is divided into three subscales (i.e., physical, cognitive, and psychosocial). The assessor records the total score of the test as the final test result. The higher the score is, the greater is the impact of fatigue in individual daily life. Therefore, the Modified Fatigue Impact Scale it is used as the description of participants' attribution of functional restrictions to fatigue symptoms. 3) Trail Making Test; It contains five conditions; the visual scanning, motor speed, number sequencing, letter sequencing, and number-letter switching. Trail Making Test also assesses attention, information processing speed and mental flexibility. This particular test consists of two parts, A and B, which involves 25 circles distributed over a sheet of paper. In Part A, the circles are numbered 1 - 25, and the participant should connect the numbers in ascending order by drawing lines. In Part B, the circles include both numbers (1 - 13) and letters (A - L); as in Part A, the participants connect the circles in an ascending pattern, but with the added task of alternating between the numbers and letters (e.g., 1-A-2-B-3-C, etc.). The participants were instructed to connect the circles as quickly as possible, without lifting the pencil from the paper. During participants' connection of the "trails", the assessor notes possible errors, and the time needed to complete the task. 4) Purdue Pegboard Test; It is a standardized test of manual dexterity. The Purdue Pegboard Test consists of four subtests, performed in a board in which pins, washers and collars are placed by the participants into two parallel columns of holes, according to the subtest task. The first two subtests are unimanual tasks, which measure dexterity of the right and left hand, respectively. The third subtest is a synchronous bimanual task that requires simultaneous use of both hands to grasp pins and place them in their

corresponding columns of holes. During the fourth subtest, the participants should perform alternating movements of both hands to complete assemblies of different types of pegs. Standard scoring of the Purdue Pegboard Test is based on the number of pegs inserted in 30 s for the first three subtests, and in 1 min for the last subtest. 5) Timed 25-Foot Walk; It is a quantitative assessment for mobility and lower limb function. Participants are directed to one end of a marked 25-foot path and they are instructed to walk as quickly as possible. The time is recorded from the start and ended when participants reached the 25-foot mark. The same task is immediately run again by having the participants walked back the same distance. Due to the fact that our participants might be using assistive devices for walking, they are instructed to use them in order to be safe when doing this task. The final score for each participant, is the mean score from the two completed trials. 6) Six Spot Step Test; It is a measure replicating a complex range of sensorimotor functions, such as lower limb strength, spasticity, coordination, as well as balance. It is a timed walking test that involves kicking over a number of targets placed along a 5 meter path. The specific test is cognitive demanding, that also includes coordination and dynamic balance.

Quality control

All scientific researchers are experienced and specialized in motor and cognitive functions in healthy individuals but also in patients with neurological and psychiatric disorders. Also, the two main organizations that work together to carry out this study are specialized in the field of rehabilitation of patients with chronic neurological diseases. The first institution is the Cyprus University of Technology and specifically the

Department of Rehabilitation Sciences of the School of Health Sciences, has a well-equipped rehabilitation clinic in which systematic studies are carried out for the rehabilitation of neurological patients. The second institution, which is the Cyprus Institute of Neurology and Genetics, is one of the most recognized centers for providing health services in Cyprus to people with chronic neurological diseases. The staff of the Neurophysiology laboratory and the Physiotherapy Unit of the Cyprus Institute of Neurology and Genetics, are specialized in evaluation and treatment services to people with chronic neurological diseases such as MS.

As depicted in the experimental procedures scheme (see Appendix 3_Figure 1), all patients began the baseline phase simultaneously and during this phase, each participant were assessed on primary and secondary outcome measures by two physiotherapists who are staff members of the Cyprus Institute of Neurology and Genetics. In order to ensure quality of the intervention a certified fitness instructor designed the protocols in collaboration with the senior physiotherapist of the Cyprus Institute of Neurology and Genetics. During the intervention phase we performed several neurophysiological (see primary outcome measures) and five clinical assessments (see secondary outcome measures) (i.e., once a week), to collect several data for every participant.

18b Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols

Due to our study design, several data were collected from the beginning of the participants' allocation. Several clinical assessments recorded for each participant. However, participants' had the opportunity to be systematically informed about their own clinical condition as well as the level of performance during the

		intervention phase. Another important factor which promoted participants' retention was the opportunity they had to exercise in a different way that they used to, as well as they offered to them a scientific exercise program under supervision without any cost.
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol All information related to the specific program, including data for all participants, collected by the principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics, who participated in the research study. From the moment each participant was enrolled to the research study he / she received a participant ID number (e.g., 101, 102). From that point on, participant's name was not mentioned and the participant ID was used. Only the principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics has access to the name of the participants. Five years after the data collection, all the material stored electronically will be permanently deleted by the principal investigator. In addition, copies of the collected data (questionnaires, clinical and cognitive tests, forms, etc.) will be immediately destroyed. All data were stored in the office of the principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics. All computers that were used in this research study for data storage were offline. In addition, logging in to computers requires an account password that was known only to the two

		previously mentioned researchers of the study. Finally, the buildings are also protected by a security company during working hours.
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol A repeated measures ANOVA analysis was conducted to determine whether there were statistically significant differences in each outcome measure across the two study groups. After conducting the analysis, we identified variables with significant differences across groups. To further explore these differences, we performed post hoc pairwise comparisons using t-tests. To control for the risk of Type I errors due to multiple comparisons, a Bonferroni correction was applied to adjust the significance *p* – value. Pearson correlation coefficient was used to determine the degree of correlation between the Symbol Digit Modalities Test and the Purdue Pegboard test. Bonferroni correction was used to determine which correlations were statistically significant. Data from the intervention phases of both groups were used to define possible relationship between the two variables, to indicate the post-exercise correlation. The null hypothesis was that "no improvement from the exercise program of the experimental group, compared to the active control group" for the outcome measure, thus participants' responses are independent from the condition (baseline vs. intervention) under which they were observed. The alternative hypothesis was that "the outcome measures of the experimental group would be affected by the specific intervention, when compared

		to the active control group". The null hypothesis was rejected if the p–value was smaller than the Bonferroni corrected p–value based on the actual number of tests that were performed (0.05/number of tests). All tests were two sided. Statistical analysis was performed using the statistical software JASPA 0.19.1 (https://jasp-stats.org/).
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) N/A due to the study design.
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) N/A due to the study design.
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed A data monitoring committee was not needed due to the study design, although a biostatistician, who was independent and blinded to the study procedures performed all statistical analysis. All data sheets which were

		completed throughout the assessments of the primary and secondary outcome measures were given to her in order to proceed with the adequate statistical analysis.
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial Due to the study design, an independent and blinded to the study procedures biostatistician performed the analysis of the data at the end of each phase (i.e., baseline, intervention, follow up). The principle investigator had access to the results but without any possibility to terminate the trial until all participants complete the intervention phase.
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct An adverse event could be defined as any unpleasant medical occurrence in a subject. Adverse events could be collected after the subject had provided consent and enrolled in the study. If a subject experience an adverse event after the informed consent document was signed but the subject had not started to perform any intervention, the event could be reported as not related to the study. All adverse events occurring during the intervention could be recorded and reported to the senior neurologist. A serious adverse event for this study could be any untoward medical occurrence that was believed by the investigators to be causally related to study intervention and results in any of the following: Life-threatening condition (that is, immediate risk of death);

		severe or permanent disability, musculoskeletal pain, fatigue. Serious adverse events occurring after a subject is discontinued from the study would NOT be reported unless the investigators feel that the event may had been caused by the exercise protocol or any of the study procedures. Investigators would determine relatedness of an event to study intervention, as well as whether the event was unexpected or unexplained given the subject's clinical course and previous clinical conditions. Due to the study design, participants were clinically examined systematically, thus there was a systematic monitoring so any possible adverse event could be reported.
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor The principal investigator and the steering committee, who were members of the entire research team, were frequently (i.e., once a week) audit the overall quality and completeness of the data, examined source documents and confirmed that all health professionals who were included in the study had complied with the requirements of the protocol. Also, they reviewed all source documents as needed, whether data sheets were completed and were updated.

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval This protocol and the template informed consent forms contained in Appendix 3 were reviewed and approved by the Cyprus National Bioethics Committee with respect to scientific content and compliance with applicable research and human subjects' regulations. The protocol, site-specific informed consent forms (local language and English versions), participant education and recruitment materials, and other requested documents also were reviewed and approved by the ethical review bodies. The principal investigator made safety and progress reports to the Cyprus National Bioethics Committee at least annually and within three months of study termination or completion.
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, patient population, sample sizes, study procedures, or significant administrative aspects could be approved by the Cyprus National Bioethics Committee prior to implementation.

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) The principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics introduced the study procedures to the participants. Participants also received information sheets and then were able to provide written consent from since are adults without any cognitive impairment. All information sheets and consent form are written in local language which is Greek).
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial Participants' study information will not be released outside of the study without the written permission of the participant. Only the principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics will have access to the name of the participants. Five years after the data collection, all the material stored electronically will be permanently deleted by the principal investigator. In addition, copies of the collected data (questionnaires, clinical and cognitive tests, forms, etc.) will be immediately destroyed.

		All data stored in the office of the principal investigator and the senior physiotherapist of the Cyprus Institute of Neurology and Genetics. All computers which were used in this research study for data storage were offline. In addition, logging in to computers requires an account password that was known only to the two previously mentioned researchers of the study. Finally, the buildings are also protected by a security company during working hours.
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site Principal investigator declared no conflict of interest.
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators The steering group had access to the full trial dataset in order to ensure the overall results. To ensure confidentiality, data dispersed to project team members were blinded of any identifying participant information.
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Patients who were enrolled into the study are covered by through the standard General Health System.

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions The scientific integrity of the project requires that all the data from all participants were analysed and reported to all team members after completion of the experimental procedures. Each paper or abstract were reviewed or/and approved by the steering committee, before being submitted to an appropriate scientific journal or/and a scientific conference. The entire research study terminated at the planned target of 1,5 years after the last participant will be enrolled to the intervention phase. Regardless of the timing and circumstances of the end of the study, close-out proceeded in two stages: - The first stage was the interim period for analysis and documentation of study results. - The second stage was the debriefing of participants and dissemination of study results, in which the paper with the final results was submitted to an appropriate journal. We expected to take about 5 to 6 months, after the last participant finished the follow up phase, to compile the final paper.
	31b	Authorship eligibility guidelines and any intended use of professional writers

		Principal investigator, steering and data manager committee were the lead authors of the entire research study. In case of some protocol authors were not named authors of subsequent publications, their role in protocol design was acknowledged in the published report.
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code No later than 2 years after the data collection and analysis, we delivered a completely data set to an appropriate data archive for sharing purposes, including published and unpublished analysis.
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates Appendix 2_ Informed Consent Form
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable N/A

Appendix 2: Informed Consent Form

CONSENT FORMS **for participation in a research program** (The forms are comprised of 10 pages)
Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

This form provides the explanations in plain and comprehensible language regarding what is being requested from you and/or what will happen to you if you agree to join the program:

12. All risks that may exist or any inconvenience you may incur from participating in the program.
13. The person(s) who will have access to your information and will arise from the program you will take part in and/or other material/data that you voluntarily provide for the program.
14. The time period during which the Principal Investigator will have access to your information and/or material concerning you.
15. What the Principal Investigator hope to learn as a result of your participation.
16. Estimation of the benefit that can be gained for researchers and/or sponsors of this program.
17. **You should not participate if you do not wish to, or if you have any concerns about your participation in the program.**
18. If you decide to join, you must indicate if you have participated in any other research programs within the last 12 months.
19. If you decide not to participate and you are a patient, your treatment will not be affected by your decision.
20. **You are free to withdraw your consent to participating in the programme at any time.**
21. If you are a patient, your decision to withdraw your consent will not have any effect on your treatment.
22. All pages of consent forms must bear your full name and signature.

Principal Investigator of the Program you are invited to participate in
Dr. Nikos Konstantinou, Assistant Professor, Department of Rehabilitation Sciences, Cyprus University of Technology. Vragadinou 15, Limassol, 3041, telephone number: 00357 25002294, email: nikos.konstantinou@cut.ac.cy

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.
Program Duration:
5 years

Do you give consent for yourself or for someone else?	
If you have responded for another person, please provide details and name.	

Question	YES or NO
Did you fill in your consent forms personally?	
Over the past 12 months, have you been involved in any other research program?	
Did you read and understand the information regarding patients and/or volunteers?	
Have you had the opportunity to ask questions and discuss the Program?	
Have you been given satisfactory answers and explanations to any of your questions?	
Do you understand that you can withdraw from the programme whenever you wish?	
Do you understand that if you withdraw, you do not need to give any explanations for your decision?	
(For patients) do you understand that, if you withdraw, there will be no impact on any treatment you get or you can get in the future?	
Do you agree to join the program?	
With whom did you speak with?	

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate

Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

Brief description of the program (procedures and purpose).

You are invited to participate in a research of the Department of Rehabilitation Sciences of the Cyprus University of Technology (CUT) in collaboration with the Cyprus Institute of Neurology and Genetics (CING). Before deciding whether or not to participate, it is important to understand the main goal of this research study. Take some time to carefully read the information below, as well as you can discuss it with others if you wish. Moreover, you can ask our team if there is anything that is not clear or you do not understand or if you would like more information about this information sheet or consent form. Take time to decide whether or not you want to participate.

The main goal of this research study, is to investigate the effects on the clinical condition and quality of life in patients with Multiple Sclerosis (MS), throughout a program of different types of exercises (i.e., in-phase bilateral exercises), which are adapted to different sports activities and fitness exercises. The study is expected to be an important tool in the implementation of future treatment programs in patients with MS. Participation in this research is voluntary. You are not expected to receive any immediate financial or personal benefit; however, your participation will greatly contribute to the development of science.

Risks of participation: There are no risks and no complications from your participation in this study.

Confidentiality: The data collected is anonymous and you do not need to provide any information about your identity. No information received will be able to identify you.

Research participation procedure: The duration of this exercise program is 12 weeks for each participant who will take part in three weekly sessions (45-60 minutes / session). Furthermore, before the beginning of the intervention, during the 12 weeks of

the intervention and in one year after the end of the intervention, there will be frequent clinical assessments of motor and cognitive functions, neurophysiological examinations (corticospinal plasticity), as well as recording of results from questionnaires concerning the quality of life and fatigue for each participant.

Right of non-participation or withdrawal: Your participation is completely voluntary and you should participate only if you wish. Choosing not to participate or leave during the research program will not have a negative effect on you, will not cost you anything and will not affect any other treatment you may receive.

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

Details of what will be requested and/or what will happen to program participants
If you agree to participate in this research study, we will ask you to come to CING. This research study includes frequent assessments before, during and after the end of a 12-week program based on sports and functional training exercises. More specifically, in the Physiotherapy Unit of ING, frequent clinical assessments and the sessions of the specific exercises will be performed based on protocol. Also, in the Neurophysiology lab of CING will be performed the neurophysiological assessments with the use of Transcranial Magnetic Stimulation (TMS). During each clinical assessment you will be asked to perform specific and very reliable motor tests, such as balance, strength, gait and functionality of the upper limbs. You will also be asked to complete a cognitive skills test for the evaluation of the information processing speed. It is important to mention that between each test you will be given enough time to rest so as to avoid fatigue. Specialist physiotherapists will record your results in each test and at the end of the program if you wish you can be given all the findings. Thru each neurophysiological assessment, measurements will be recorded in relation to the corticospinal plasticity of the two cerebral hemispheres and the central motor conduction time of the upper limbs via TMS. At this point we will ask you to sit comfortably in an armchair in order to activate the motor cortex of the brain. Using special electrodes that will be placed in both hands, we will monitor the activity of a muscle in each hand respectively. When applying TMS, we will activate your brain cells with simple magnetic pulses produced by an insulated coil which we will place on your scalp. Each pulse travels through your scalp, causing a small electric current in the cortex (the outer part of the brain). The goal is to find the area of the brain that corresponds to the specific muscle of the hand in which we have placed the special electrode. It is important to know that magnetic pulses can cause a slight tingling

sensation on your scalp. This sensation is usually not unpleasant but sometimes it can actually cause an annoying sensation.

The exercise program in which you will be invited to take part, includes exercises that contain in-phase bilateral movements and are adapted to different sports activities and functional exercises. All exercises will be under the guidance and supervision of specialized sport scientist and physiotherapists experienced in the field of neurorehabilitation and you will need to wear the appropriate sportswear.

The purpose of this research study is to investigate the effect of specific exercises on your clinical condition as well as on the functioning of the central nervous system. It is important to know that you can request to stop and leave this program at any time without any excuse and with no consequences.

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

Details of any risks that may exist or any inconvenience that program participants may incur
By providing a series of exercises for a long time, you may feel tired. In case this happens you will be given specific guidelines in order to recover. All researchers are health professionals and experienced in the field of neurorehabilitation and exercising in chronic diseases.

Details of what information and/or what material will be collected under the program, who will have access to it and for how long.
You will not be asked for any personal data that could lead to your identification. During the program, all data will be collected in relation to your motor and cognitive condition, as well as your motor cortex activity via TMS. At the same time, you will be given the Safety Check Form for TMS, which you will be asked to state if you have a history of specific diseases.

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

WHERE APPLICABLE, FUTURE STORAGE AND USE OF BIOLOGICAL SAMPLES AND PERSONAL DATA: Please note and sign either left or right	
Except for the purposes of this program that will last for ... . years **I consent**: ☐ Signature:	Except for the purposes of this study that will last for ... . years **I do not consent**: ☐ Signature:
that my biological samples (buccal swabs, saliva or DNA) and genetic information which shall be stored at the …… **<u>may be kept for more than …… years and be used in future studies</u>** upon authorization of the Cyprus National Bioethics Committee (CNBC), following the relevant application for renewal by the Principal Investigator of this Program, I understand that matters of confidentiality will always be in force.	

If new information that directly affects your health is discovered, would you like to be informed?		
YES ☐	NO ☐	I CANNOT MAKE A DECISION NOW. PLEASE ASK AGAIN IF NEEDED ☐

Details of what data will be generated for you within the program, who will have access to them and for how long.
All data that will be gained, are related to motor and cognitive skills, as well as to the motor cortex activity. Only the research team will have access to this data and it will be destroyed after 5 years. In case of publication of the results of the present study in a scientific journal or in any other conference, any of your personal data will not be published and you will not be able to identify yourself with any published material.

Surname:	……	Name:	……
Signature:	……	Date:	……

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.
Expected benefit for participants
The benefits that you will have with your participation in this research study is the systematic evaluation of your clinical condition for a year, as well as your participation for a long time in an exercise program that will keep you physically active with possible improvement of your motor and cognitive functions and improving your quality of life.
Expected benefit for researchers and/or sponsors
All results from the study, will contribute to the scientific knowledge but also to the practical application by health professionals, regarding the effect of this type of movements and exercises in order to improve the current clinical situation of people with MS. Moreover, they will offer the advantage for better quality of rehabilitation in possible future progression of the disease.
Details of termination or early postponement of the research program.
All data that will collected from your assessments will be stored in the laboratory of the rehabilitation clinic, at the School of Health Sciences, of the CUT, as well as in the office of the senior physiotherapist of the Physiotherapy Unit, of CING. All the data that will be received within the specific research program will be destroyed after 5 years.
Description of procedures of handling data and/or biological samples of participants who withdraw from the study prior to its completion.
The data of the participants who will withdraw from the study before its completion will be destroyed immediately and will not be used in any study process. Specifically, any data stored on the researchers' computers will be deleted when you leave the study by the PI and any printed material collected will be destroyed and recycled.

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

CONSENT FORMS

for participation in a research program

(The forms are comprised of 10 pages)

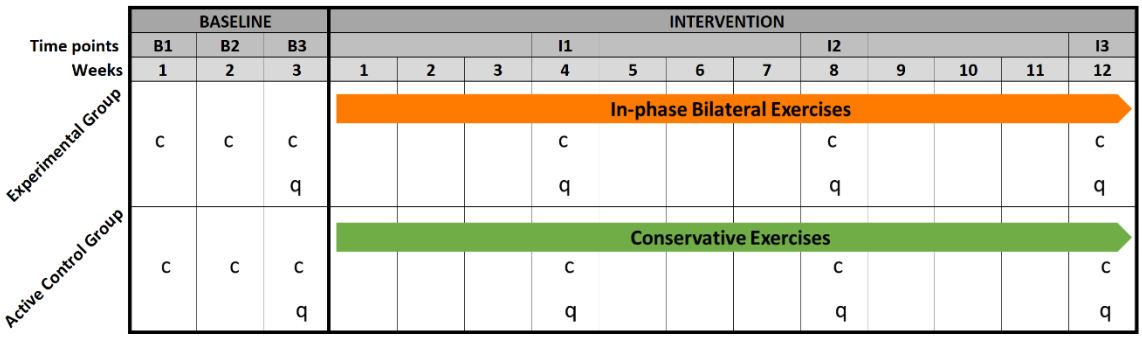
Title of the Programme you are invited to participate
Investigation of in-phase bilateral exercise effects in people with multiple sclerosis.

Full contact details and title of the person to whom participants can submit complaints or grievances regarding the program they participate in.
Dr. Charalambos Charalambous Head of the department of research and international collaborations Cyprus University of Technology Telephone number: 00357 25 002562 email: c.chrisostomou@cut.ac.cy

Full contact details and title of the person whom participants can contact for more information or clarifications about the research program.
Dr. Nikos Konstantinou Assistant Professor, Department of Rehabilitation Sciences, Cyprus University of Technology. Vragadinou 15, Limassol, 3041 telephone number: 00357 25002294 email: nikos.konstantinou@cut.ac.cy

Surname:	.....	Name:	.....
Signature:	.....	Date:	.....

Appendix 3: Figure 1



Note: B; baseline, I; intervention, c; clinical assessments, q; questionaries. The first row refers to the study phases (i.e., baseline and intervention). The second row refers to the time points of the data collection. The third row refers to the study weeks. The present study had a 15-weeks duration in total, form which the first three weeks refers to the baseline phase and the rest 12 weeks refer to the intervention phase. During the intervention phases, the participants in the experimental group performed an exercise program based on in-phase bilateral exercises of the upper limbs (orange color), while those in the active control group performed conventional exercises (green color).

APPENDIX XI

Examples of conventional exercises

Figure 1. Trunk stabilization exercises.

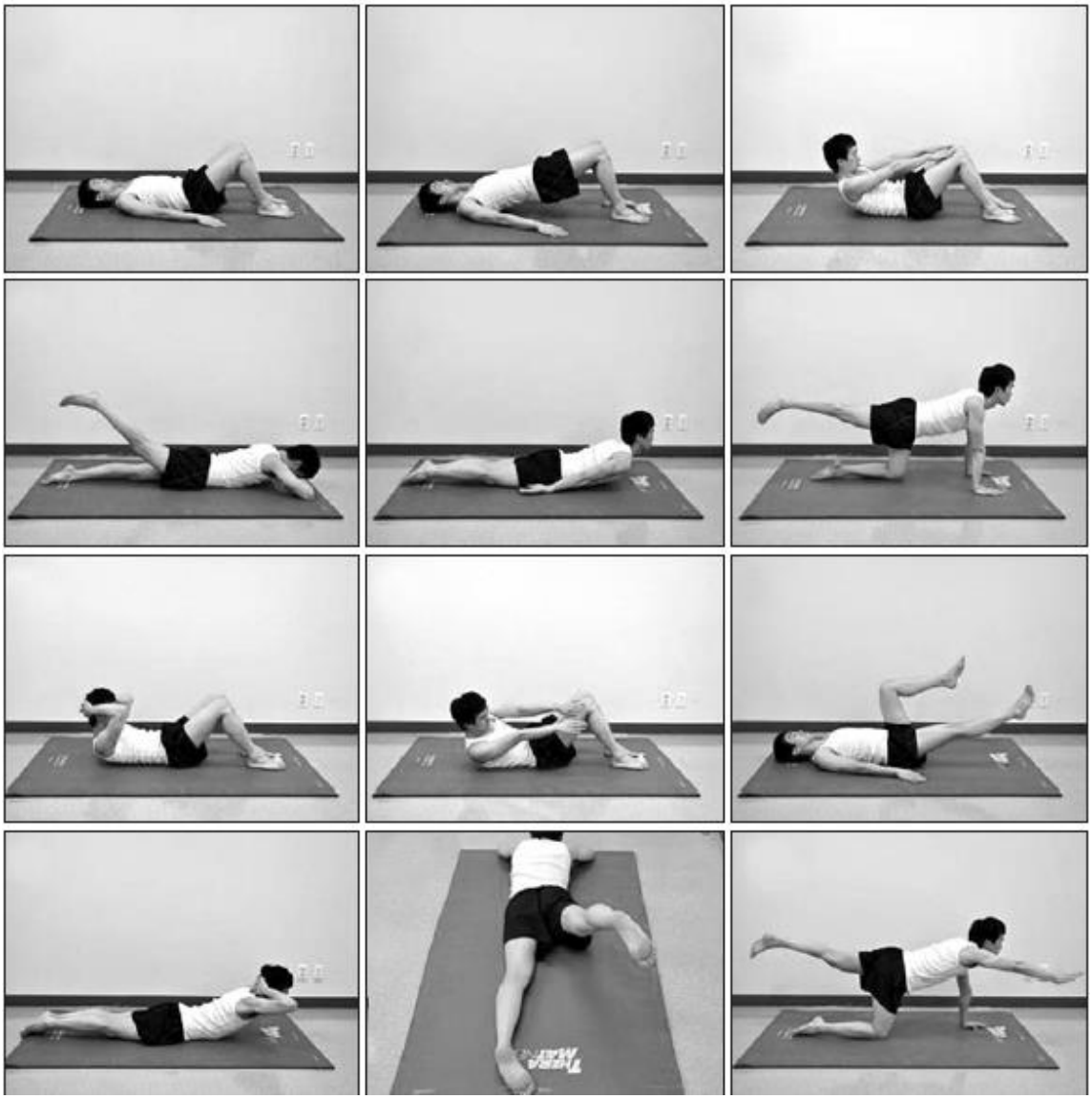


Figure 2. Advanced trunk stabilization exercises.

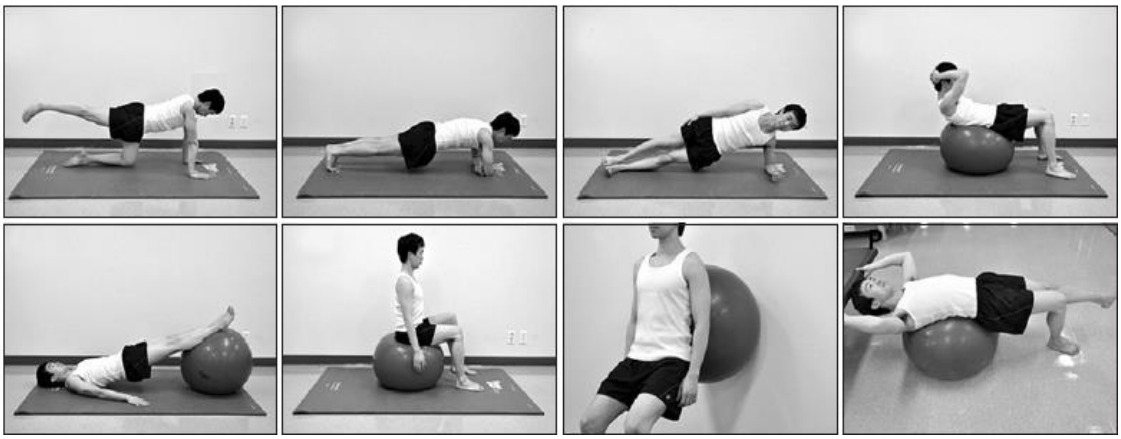
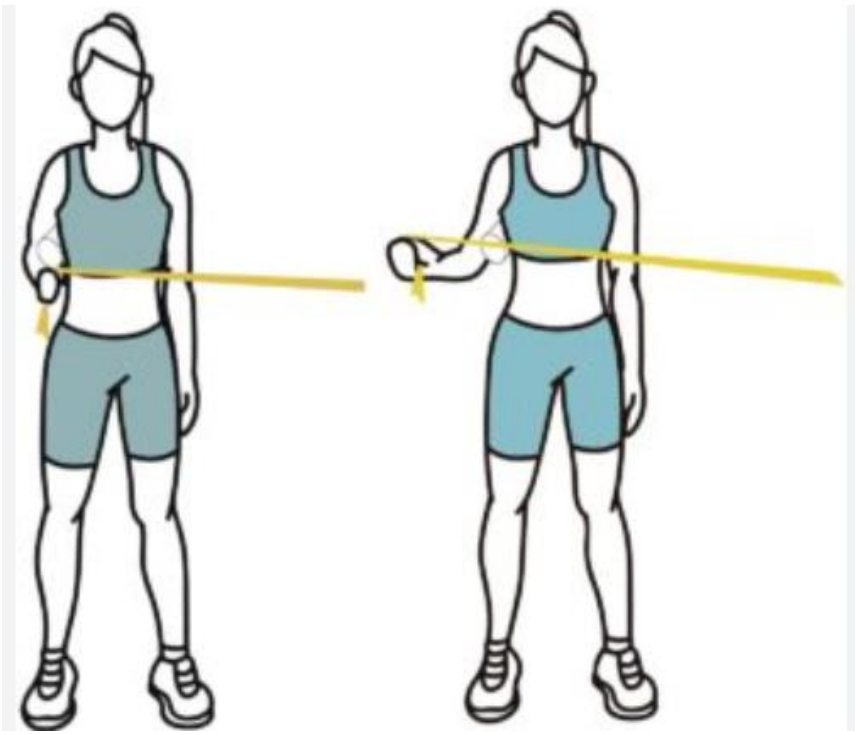
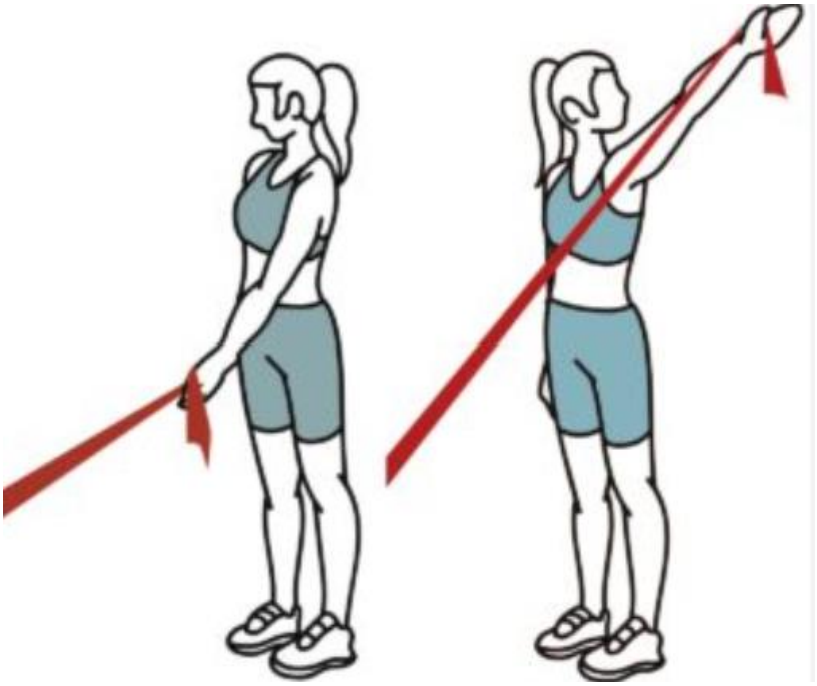


Figure 3. Upper limbs – Resistance exercises.

a.



b.



c.

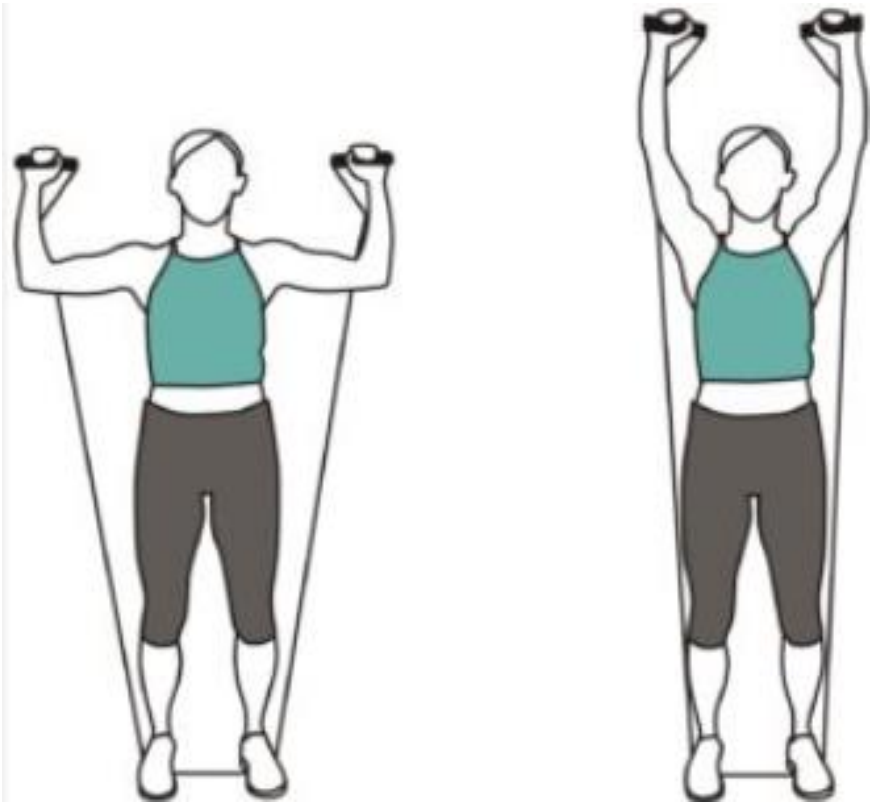


Figure 4. Aerobic exercise – Treadmill.

Note: To ensure the participants' safety, they were all fitted with a special jacket that was integrated into a harness for added security.

Figure 5. Aerobic exercise – MOTOmed Loop Parkinson.

APPENDIX XII

Group Comparison – Individual results based on the outcome measure and group allocation.

Table 1. Symbol Digit Modalities Test groups data across all assessment points during baseline and intervention phases.

Experimental Group									
Participant	**B1**	**B2**	**B3**	**Mean**	**I1**	**I2**	**I3**	**Mean**	**Improve**
1	47 (0)	45 (0)	45 (0)	45.6 (0)	50 (0)	52 (0)	51 (0)	51 (0)	5.3 (0)
2	51 (0)	51 (0)	52 (0)	51.3 (0)	57 (0)	56 (0)	56 (0)	56.3 (0)	5 (0)
3	55 (0)	54 (0)	56 (0)	55 (0)	62 (0)	64 (0)	63 (0)	63 (0)	8 (0)
4	44 (0)	44 (0)	43 (0)	43.6 (0)	50 (0)	51 (0)	52 (0)	51 (0)	7.3 (0)
5	69 (0)	70 (0)	69 (0)	69.3 (0)	74 (0)	73 (0)	74 (0)	73.6 (0)	4.3 (0)
6	74 (0)	73 (0)	74 (0)	73.6 (0)	79 (0)	81 (0)	80 (0)	80 (0)	6.3 (0)
7	72 (0)	75 (0)	71 (0)	72.6 (0)	80 (0)	81 (0)	80 (0)	80.3 (0)	7.6 (0)
8	57 (0)	58 (0)	58 (0)	57.6 (0)	63 (0)	65 (0)	64 (0)	64 (0)	6.3 (0)
9	52 (0)	50 (0)	53 (0)	51.6 (0)	59 (0)	59 (0)	60 (0)	59.3 (0)	7.6 (0)

10	32 (0)	30 (0)	29 (0)	30.3 (0)	35 (0)	36 (0)	34 (0)	35 (0)	4.6 (0)
Mean	**55.3 (0)**	**55 (0)**	**55 (0)**	**55.1 (0)**	**60.9 (0)**	**61.8 (0)**	**61.4 (0)**	**61.3 (0)**	**6.2 (0)**
SD	**13.2 (0)**	**14.3 (0)**	**13.9 (0)**	**13.8 (0)**	**14.1 (0)**	**14.1 (0)**	**14.2 (0)**	**14.1 (0)**	**1.4 (0)**
Active Control Group									
1	50 (0)	51 (0)	50 (0)	50.3 (0)	53 (0)	51 (0)	52 (0)	52 (0)	1.6 (0)
2	36 (0)	35 (0)	34 (0)	35 (0)	38 (0)	36 (0)	37 (0)	37 (0)	2 (0)
3	35 (0)	36 (0)	36 (0)	35.6 (0)	37 (0)	39 (0)	39 (0)	38.3 (0)	2.6 (0)
4	59 (0)	60 (0)	59 (0)	59.3 (0)	61 (0)	60 (0)	62 (0)	61 (0)	1.6 (0)
5	64 (0)	64 (0)	65 (0)	64.3 (0)	65 (0)	66 (0)	68 (0)	66.3 (0)	2 (0)
6	70 (0)	71 (0)	70 (0)	70.3 (0)	72 (0)	73 (0)	71 (0)	72 (0)	1.6 (0)
7	69 (0)	69 (0)	68 (0)	68.6 (0)	70 (0)	71 (0)	73 (0)	71.3 (0)	2.6 (0)
8	45 (0)	46 (0)	46 (0)	45.6 (0)	49 (0)	48 (0)	49 (0)	48.6 (0)	3 (0)
9	53 (0)	52 (0)	53 (0)	52.6 (0)	54 (0)	54 (0)	55 (0)	54.3 (0)	1.6 (0)

10	68 (0)	68 (0)	69 (0)	68.3 (0)	72 (0)	70 (0)	72 (0)	71.3 (0)	3 (0)
Mean	**54.9 (0)**	**55.2 (0)**	**55 (0)**	**55 (0)**	**57.1 (0)**	**56.8 (0)**	**57.8 (0)**	**57.2 (0)**	**2.2 (0)**
SD	**13.2 (0)**	**13.3 (0)**	**13.4 (0)**	**13.3 (0)**	**13.1 (0)**	**13.3 (0)**	**13.4 (0)**	**13.2 (0)**	**0.5 (0)**

Table 1: B; Baseline, I; Intervention, SD; Standard Deviation. Values were expressed in numbers, numbers in briefcases refer to the mistakes which they were done in each assessment point. Improvement was calculated as the difference between B and mean I (i.e., Improve = mean Intervention – mean Baseline).

Table 2. Medical Outcomes Study Questionnaire Short Form 36 Health Survey groups data across all assessment points during baseline and intervention phases.

Experimental Group						
Participant	**B**	**I1**	**I2**	**I3**	**Mean I**	**Improve**
1	81	83	87	88	86	5
2	82	87	86	90	87.6	5.6
3	85	92	94	94	93.3	8.3
4	91	98	99	101	99.3	8.3
5	85	90	92	97	93	8
6	100	103	104	105	104	4
7	93	99	104	105	102.6	9.6
8	94	99	97	99	98.3	4.3
9	85	94	95	97	95.3	10.3
10	92	94	95	100	96.3	4.3
Mean	**88.8**	**93.9**	**95.3**	**97.6**	**95.6**	**6.8**
SD	**6.1**	**5.8**	**5.7**	**5.4**	**5.5**	**2.3**

Active Control Group						
1	87	86	90	93	89.6	2.6
2	88	87	83	86	85.3	-2.6
3	81	81	81	80	80.6	-0.3
4	79	80	78	77	78.3	-0.6
5	81	81	83	82	82	1
6	101	103	103	76	94	-7
7	72	73	72	77	74	2
8	87	88	88	87	87.6	0.6
9	96	98	97	96	97	1
10	85	85	88	90	87.6	2.6
Mean	**85.7**	**86.2**	**86.3**	**84.4**	**85.6**	**0**
SD	**8.3**	**8.3**	**8.5**	**6.7**	**6.7**	**2.9**

Table 2: B; Baseline, I; Intervention, SD; Standard Deviation. Values were expressed in numbers. Improvement was calculated as the difference between B and mean I Improvement was calculated as the difference between B and mean I (i.e., Improve = mean Intervention – mean Baseline). All negative values indicate no individual improvement.

Table 3. Modified Fatigue Impact Scale groups data across all assessment points during baseline and intervention phases.

Experimental Group						
Participant	**B**	**I1**	**I2**	**I3**	**Mean I**	**Improve**
1	34	30	30	31	30.3	3.6
2	52	49	48	48	48.3	3.6
3	28	21	15	16	17.3	10.6
4	14	12	10	4	8.6	5.3
5	53	46	43	43	44	9

6	43	18	15	15	16	27
7	34	26	15	3	14.6	19.3
8	16	15	14	11	13.3	2.6
9	44	30	27	27	28	16
10	56	50	46	45	47	9
Mean	**34**	**29.7**	**26.3**	**24.3**	**26.7**	**10.6**
SD	**14.8**	**14.1**	**14.7**	**16.9**	**15**	**7.9**
Active Control Group						
1	49	50	52	52	51.3	-2.3
2	48	48	49	50	49	-1
3	81	82	80	81	81	0
4	23	24	22	24	23.3	-0.3
5	66	65	66	66	65.6	0.3
6	48	47	48	49	48	0
7	35	34	35	29	32.6	2.3
8	26	29	27	26	27.3	-1.3
9	17	17	16	16	16.3	0.6
10	55	55	59	62	58.6	-3.6
Mean	**44.8**	**50**	**52**	**52**	**45.3**	**-0.5**
SD	**19.9**	**19.7**	**20.3**	**21.1**	**20.3**	**1.6**

Table 3: B; Baseline, I; Intervention, SD; Standard Deviation. Values were expressed in numbers. Improvement was calculated as the difference between B and mean I Improvement was calculated as the difference between B and mean I (i.e., Improve = mean Intervention – mean Baseline). All negative values indicate no individual improvement.

Table 4. Purdue Pegboard Test - Unimanual dominant hand subtest, groups data across all assessment points during baseline and intervention phases.

Experimental Group									
Participant	**B1**	**B2**	**B3**	**Mean**	**I1**	**I2**	**I3**	**Mean**	**Improve**
1	8	8	8	8	9	9	9	9	1
2	14	13	14	13.6	15	16	16	15.6	2
3	11	12	11	11.3	15	16	16	15.6	4.3
4	13	13	12	12.6	15	15	15	15	2.4
5	14	13	14	13.6	15	16	16	15.6	2
6	16	16	15	15.6	16	16	17	16.3	0.7
7	13	12	13	12.6	14	14	14	14	1.4
8	13	13	12	12.6	15	16	15	15.3	2.7
9	13	14	13	13.3	15	14	15	14.6	1.4
10	9	8	9	8.6	10	12	12	11.3	2.7
Mean	**12.4**	**12.2**	**12.1**	**12.2**	**13.9**	**14.4**	**14.5**	**14.2**	**2**
SD	**2.4**	**2.4**	**2.2**	**2.3**	**2.3**	**2.3**	**2.3**	**2.3**	**1**
Active Control Group									
1	10	10	11	10.3	9	10	9	9.3	-1
2	8	8	8	8	8	9	8	8.3	0.3
3	11	11	9	10.3	12	10	11	11	0.7
4	8	8	9	8.3	9	10	10	9.6	1.3
5	13	12	14	13	11	12	12	11.6	-1.4
6	12	10	10	10.6	12	10	10	10.6	0
7	13	13	14	13.3	12	12	12	12	-1.7
8	14	13	11	12.6	13	13	13	13	0.4

9	8	8	12	9.3	9	10	11	10	0.7
10	11	12	11	11.3	10	9	11	10	-1.3
Mean	**10.8**	**10.5**	**10.9**	**10.7**	**10.5**	**10.5**	**10.7**	**10.5**	**-0.2**
SD	**2.2**	**2**	**2**	**1.8**	**1.7**	**1.3**	**1.4**	**1.3**	**0.9**

Table 4: B; Baseline, I; Intervention, SD; Standard Deviation. Values were expressed in numbers. Improvement was calculated as the difference between B and mean I Improvement was calculated as the difference between B and mean I (i.e., Improve = mean Intervention – mean Baseline). All negative values indicate no individual improvement.

Table 5. Purdue Pegboard Test - Unimanual non-dominant hand subtest, groups data across all assessment points during baseline and intervention phases.

Experimental Group									
Participant	**B1**	**B2**	**B3**	**Mean**	**I1**	**I2**	**I3**	**Mean**	**Improve**
1	8	7	6	7	7	7	7	7	0
2	9	9	9	9	9	10	11	10	1
3	11	11	10	10.6	12	12	11	11.6	1
4	11	10	10	10.3	15	13	14	14	3.7
5	13	13	13	13	15	14	14	14.3	1.3
6	13	14	13	13.3	14	14	14	14	0.7
7	11	10	11	10.6	12	13	12	12.3	1.7
8	12	11	11	11.3	12	12	14	12.6	1.3
9	10	10	11	10.3	11	12	12	11.6	1.3
10	7	7	8	7.3	10	10	11	10.3	3
Mean	**10.5**	**10.2**	**10.2**	**10.3**	**11.7**	**11.7**	**12**	**11.8**	**1.5**
SD	**2**	**2.2**	**2.1**	**2**	**2.5**	**2.1**	**2.2**	**2.2**	**1**
Active Control Group									
1	7	8	8	7.6	9	7	6	7.3	-0.3

2	7	7	8	7.3	8	8	8	8	0.7
3	7	8	7	7.3	8	7	8	7.6	0.3
4	6	5	9	6.6	8	10	9	9	2.4
5	11	11	10	10.6	10	9	10	9.6	-1
6	10	11	9	10	11	10	9	10	0
7	13	12	14	13	10	10	8	9.3	-3.7
8	12	13	10	11.6	12	9	10	10.3	-1.3
9	6	7	7	6.6	8	8	9	8.3	1.6
10	10	11	11	10.6	10	9	6	8.3	-2.3
Mean	**8.9**	**9.3**	**9.3**	**9.1**	**9.4**	**8.7**	**8.3**	**8.8**	**-0.3**
SD	**2.6**	**2.6**	**2.1**	**2.3**	**1.4**	**1.1**	**1.4**	**1**	**1**

Table 5: B; Baseline, I; Intervention, SD; Standard Deviation. Standard Deviation. Values were expressed in numbers. Improvement was calculated as the difference between B and mean I Improvement was calculated as the difference between B and mean I (i.e., Improve = mean Intervention – mean Baseline). All negative values indicate no individual improvement.

Table 6. Purdue Pegboard Test – Bimanual subtest, groups data across all assessment points during baseline and intervention phases.

Experimental Group									
Participant	**B1**	**B2**	**B3**	**Mean**	**I1**	**I2**	**I3**	**Mean**	**Improve**
1	6	7	8	7	5	7	8	6.6	-0.4
2	8	7	9	8	10	10	10	10	2
3	8	10	10	9.3	11	11	11	11	1.6
4	9	10	11	10	10	11	12	11	1
5	10	10	10	10	12	12	13	12.3	2.3
6	10	10	11	10.3	12	13	13	12.6	2.3
7	10	11	11	10.6	12	13	11	12	1.4

8	12	9	12	11	10	13	14	12.3	1.3
9	10	10	10	10	11	11	15	12.3	2.3
10	7	8	8	7.6	10	11	12	11	3.3
Mean	**9**	**9.2**	**10**	**9.4**	**10.3**	**11.2**	**11.9**	**11.1**	**1.7**
SD	**1.7**	**1.3**	**1.8**	**1.3**	**2**	**1.8**	**2**	**1.7**	**0.9**
Active Control Group									
1	6	9	8	7.6	8	7	7	7.3	-0.3
2	6	7	5	6	8	7	6	7	1
3	5	7	8	6.6	9	9	9	9	2.4
4	9	9	11	9.6	8	12	10	10	0.4
5	10	10	10	10	9	10	10	9.6	-0.4
6	8	9	9	8.6	8	8	9	8.3	-0.3
7	11	10	10	10.3	10	10	8	9.3	-1
8	10	10	11	10.3	11	10	9	10	-0.3
9	6	7	8	7	9	9	7	8.3	1.3
10	10	9	10	9.6	10	10	11	10.3	0.6
Mean	**8.1**	**8.7**	**9**	**8.6**	**9**	**9.2**	**8.6**	**8.9**	**0.3**
SD	**2.1**	**1.2**	**1.8**	**1.6**	**1**	**1.5**	**1.5**	**1.1**	**1**

Table 6: B; Baseline, I; Intervention, SD; Standard Deviation. Values were expressed in numbers. Improvement was calculated as the difference between B and mean I Improvement was calculated as the difference between B and mean I (i.e., Improve = mean Intervention – mean Baseline). All negative values indicate no individual improvement.

Table 7. Purdue Pegboard Test – Assembly subtest, groups data across all assessment points during baseline and intervention phases.

Experimental Group								
Participant	**B1**	**B2**	**B3**	**Mean**	**I1**	**I2**	**I3**	**Mean**	**Improve**

1	8	12	10	10	12	15	14	13.6	3.6
2	17	15	16	16	17	18	17	17.3	1.3
3	18	18	18	18	20	24	24	22.6	4.6
4	23	20	21	2.3	24	25	25	24.6	3.3
5	17	18	20	18.3	25	27	26	26	7.7
6	24	25	25	24.6	30	31	32	31	6.4
7	16	15	18	16.3	22	23	21	22	5.7
8	16	18	19	17.6	24	24	24	24	6.4
9	24	21	22	22.3	25	25	28	26	3.7
10	11	12	12	11.6	14	16	15	15	3.4
Mean	**17.4**	**17.4**	**18.1**	**17.6**	**21.3**	**22.8**	**22.6**	**22.3**	**4.7**
SD	**5.2**	**4**	**4.5**	**4.5**	**5.5**	**5**	**5.8**	**5.4**	**1.8**
Active Control Group									
1	16	15	16	15.6	15	16	15	15.3	-0.3
2	12	13	12	12.3	12	15	14	13.6	1.3
3	16	13	18	15.6	15	15	16	15.3	-0.3
4	11	10	15	12	13	12	13	12.6	0.6
5	15	14	13	14	15	14	16	15	1
6	14	13	17	14.6	14	12	16	14	-0.6
7	24	23	21	22.6	21	21	24	22	-0.6
8	18	19	15	17.3	20	17	17	18	0.7
9	10	9	10	9.6	11	11	11	11	1.4
10	15	10	16	13.6	13	14	15	14	0.4
Mean	**15.1**	**13.9**	**15.3**	**14.7**	**14.9**	**14.7**	**15.7**	**15.1**	**0.4**
SD	**3.9**	**2**	**2.6**	**3.5**	**3.2**	**2.9**	**3.4**	**3**	**0.7**

Table 7: B; Baseline, I; Intervention, SD; Standard Deviation. Values were expressed in numbers. Improvement was calculated as the difference between B and mean I (i.e., Improve = mean Intervention – mean Baseline). All negative values indicates no individual improvement.

Table 8. Purdue Pegboard Subtests - improvement between groups.

Group	Unimanual Dominant	Unimanual Non-dominant	Bimanual	Assembly
Experimental	2.03	1.5	1.73	4.6
SD	1.05	1.08	0.99	1.89
Active Control	-0.16	-0.36	0.33	0.33
SD	0.99	1.8	1	0.78

Table 8: SD; Standard Deviation. Values were expressed in numbers. Improvement was calculated as the mean difference from each subtest between B and I (i.e., mean Intervention – mean Baseline). All negative values indicates no individual improvement.

Table 9. Timed 25-Foot Walk Test groups data across all assessment points during basely and intervention phases.

Experimental Group									
Participant	**B1**	**B2**	**B3**	**Mean**	**I1**	**I2**	**I3**	**Mean**	**Improve**
1	10.4	10.9	10.3	10.5	9.6	8.1	7.8	8.5	2
2	7.4	7.9	7.2	7.5	5.6	5.6	5.6	5.6	1.9
3	9.9	9.5	10.3	9.9	5.7	6.7	6.4	6.3	3.6
4	6.7	6.7	6.7	6.7	5.5	5.1	5.1	5.2	1.5
5	7.3	7.6	7.5	7.5	6.8	6	5.2	6	1.5
6	8.7	9.3	9.2	9	7.4	6.5	6.5	6.8	2.2
7	8.2	8.7	8.4	8.4	6.6	6.9	6.1	6.5	1.9
8	6.8	6.6	7.1	6.8	5.8	5	5.1	5.3	1.5

9	9.4	8.4	9.1	8.9	6.3	4.9	4.9	5.4	3.5
10	14.5	14.1	14.8	14.4	12.2	12.2	11.3	11.9	2.5
Mean	**8.9**	**8.9**	**9**	**9**	**7.1**	**6.7**	**6.4**	**6.7**	**2.3**
SD	**2.3**	**2.2**	**2.3**	**2.3**	**2.1**	**2.1**	**1.9**	**2**	**0.7**
Active Control Group									
1	11.9	11.7	12.4	12	12.4	12.7	12.4	12.5	-0.5
2	12	12	11.2	11.7	11.7	12.5	10.2	11.5	0.2
3	13.4	13.6	13.3	13.4	13.9	13.5	13.7	13.7	-0.3
4	7.7	8	7.6	7.8	8.3	7.7	7.5	7.8	0
5	6.7	6.7	6.6	6.6	6.5	7	6.7	6.7	0
6	8.4	8.6	8.2	8.4	8.6	8.1	8	8.2	0.2
7	5.5	6.2	5.8	5.8	5.6	6.1	5.5	5.7	0
8	6.6	6.7	6.6	6.6	6.6	6.7	6.5	6.6	0
9	10.5	10.7	10.3	10.5	10.5	10.9	10.4	10.6	0.1
10	11.6	11.8	11.6	11.6	11.9	12.1	11.3	11.8	-0.2
Mean	**9.4**	**9.6**	**9.3**	**9.4**	**9.6**	**9.7**	**9.2**	**9.5**	**0.1**
SD	**2.7**	**2.6**	**2.7**	**2.7**	**2.8**	**2.8**	**2.7**	**2.8**	**0.2**

Table 9: B; Baseline, I; Intervention, SD; Standard Deviation. Values were expressed in numbers. Improvement was calculated as the difference between B and mean I Improvement was calculated as the difference between B and mean I (i.e., Improve = mean Intervention – mean Baseline). All negative values indicate no individual improvement.

Table 10. Six Spot Step Test groups data across all assessment points during baseline and intervention phases.

Experimental Group								
Participant	**B1**	**B2**	**B3**	**Mean**	**I1**	**I2**	**I3**	**Mean**	**Improve**
1	29.4	31.8	29.2	30.2	26	22.8	22.6	23.8	6.4

2	26.4	28.4	28.1	27.6	23.1	23	22.9	23	4.6
3	9.6	9.9	9.6	9.7	7.5	7.7	7.6	7.6	2.1
4	7.8	8.1	7.8	7.9	6.8	6.4	6.2	6.5	1.4
5	10.5	10.3	10.3	10.4	8.7	8.2	8.4	8.4	2
6	7	6.9	6.9	6.9	5.9	5.1	5.6	5.5	1.4
7	11.1	11.7	11.6	11.5	10.5	10.2	10.3	10.3	1.2
8	10.9	10.9	11.4	11.1	10	9.8	10	9.9	1.2
9	7.7	8	8.	7.9	6.8	6.6	6	6.5	1.4
10	10.1	10.8	10.3	10.4	9	8.8	8.4	8.7	1.7
Mean	**13**	**13.7**	**13.3**	**13.3**	**11.4**	**10.8**	**10.8**	**11**	**2.3**
SD	**7.9**	**8.8**	**8.2**	**8.3**	**7**	**6.5**	**6.4**	**6.6**	**1.7**
Active Control Group									
1	36.1	36.5	36.4	36.3	37.2	37.1	37.1	37.1	-0.8
2	19.5	20	19.7	19.7	19.8	19.6	20.1	19.8	0.1
3	8.7	8.4	8.6	8.5	8.9	8.4	8.7	8.7	-0.2
4	19.1	18.8	19.3	19	19.1	18.9	19.1	19	0
5	22.8	23.2	23	23	23.1	23.3	22.9	23.1	-0.1
6	7.4	7.6	7.4	7.5	7.8	7.5	7.6	7.6	-0.1
7	11.7	11.7	11.8	11.7	11.9	11.5	11.7	11.7	0
8	10.2	10.3	10.4	10.3	10.3	10.1	10.6	10.3	0
9	10.3	10.5	10.7	10.5	10.3	10.4	10.7	10.5	0
10	17.3	17.3	17.7	17.4	17.4	17.6	18	17.7	-0.3
Mean	**16.3**	**16.4**	**16.5**	**16.4**	**16.6**	**16.4**	**16.6**	**16.5**	**-0.1**
SD	**8.7**	**8.8**	**8.7**	**8.7**	**8.9**	**9**	**8.9**	**8.9**	**0.2**

Table 10: B; Baseline, I; Intervention, SD; Standard Deviation. Values were expressed in numbers. Improvement was calculated as the difference between B and mean I Improvement was calculated as the difference between B and mean I (i.e., Improve = mean Intervention – mean Baseline). All negative values indicate no individual improvement.

APPENDIX XIII

ANOVA analysis

Symbol Digit Modalities Test

Table 1. Symbol Digit Modalities Test – Within Subjects Effects.

Cases	F	*p*
Study Phases	326.20	< 0.001
Study Phases ✻ Group	75.25	< 0.001

Note: ✻; Interaction between the two variables.

Table 2. Symbol Digit Modalities Test – Post Hoc Comparisons – Study Phases.

		Mean Difference	SE	t	p_{bonf}
Baseline	Intervention	-4.23	0.23	-18.06	< 0.001

Table 3. Symbol Digit Modalities Test – Post Hoc Comparisons – Group ✻ Study Phases.

		Mean Difference	SE	t	p_{bonf}
Experimental, Baseline	Active, Control, Baseline	0.06	6.07	0.01	1.000
	Experimental, Intervention	-6.26	0.33	-18.9	< 0.001
	Active, Control, Intervention	-2.13	6.1	-0.35	1.000
Active, Control, Baseline	Experimental, Intervention	-6.33	6.1	-1.03	1.000
	Active, Control, Intervention	-2.2	0.33	-6.63	< 0.001
Experimental, Intervention	Active, Control, Intervention	4.13	6.14	0.67	1.000

Note: Results are averaged over the levels of: Assessments Points.

Table 4. Symbol Digit Modalities Test – *t*-test – Improvement difference between groups.

	Experimental Group	**Active Control Group**
Mean	6.2	2.2
Observations	10	10
df	18	
t statistic	8.6	
p - value	< 0.05	

Medical Outcomes Study Questionnaire Short Form 36 Health Survey

Table 5. Medical Outcomes Study Questionnaire Short Form 36 Health Survey – Within Subjects Effects.

Cases	**F**	***p***
Study Phases	4.86	0.005
Study Phases ✻ Group	7	< 0.001

Note: ✻; Interaction between the two variables.

Table 6. Medical Outcomes Study Questionnaire Short Form 36 Health Survey – Post Hoc Comparisons – Study Phases.

		Mean Difference	**SE**	**t**	p_{bonf}
Baseline	I1	-2.8	0.39	-7.01	< 0.001
	I2	-3.55	0.59	-5.93	< 0.001
	I3	-3.75	1.46	-2.56	0.117
I1	I2	-0.75	0.49	-1.5	0.897
	I3	-0.95	1.53	-0.61	1.000

I2	I3	-0.2	1.46	-0.13	1.000

Table 7. Medical Outcomes Study Questionnaire Short Form 36 Health Survey – Post Hoc Comparisons - Group ✻ Study Phases.

		Mean Difference	**SE**	**t**	p_{bonf}
Experimental, Baseline	Active, Control, Baseline	3.1	3.27	0.94	1.000
	Experimental, I1	-5.1	0.56	-9.03	< 0.001
	Active, Control, I1	2.6	3.32	0.78	1.000
	Experimental, I2	-6.5	0.84	-7.68	< 0.001
	Active, Control, I2	2.5	3.36	0.74	1.000
	Experimental, I3	-8.8	2.06	-4.25	0.013
	Active, Control, I3	4.4	3.08	1.42	1.000
Active, Control, Baseline	Experimental, I1	-8.2	3.32	-2.46	0.672
	Active, Control, I1	-0.5	0.56	-0.88	1.000
	Experimental, I2	-9.6	3.36	-2.85	0.294
	Active, Control, I2	-0.6	0.84	-0.71	1.000
	Experimental, I3	-11.9	3.08	-3.86	0.032
	Active, Control, I3	1.3	2.06	0.62	1.000
Experimental, I1	Active, Control, I1	7.7	3.38	2.27	0.99
	Experimental, I2	-1.4	0.7	-1.98	1.000
	Active, Control, I2	7.6	3.41	2.22	1.000
	Experimental, I3	-3.7	2.17	-1.7	1.000

	Active, Control, I3	9.5	3.1	3.02	0.2
Active, Control, I1	Experimental, I2	-9.1	3.4	-2.66	0.44
	Active, Control, I2	-0.1	0.7	-0.14	1.000
	Experimental, I3	-11.4	3.14	-3.62	0.054
	Active, Control, I3	1.8	2.17	0.82	1.000
Experimental, I2	Active, Control, I2	9	3.45	2.6	0.49
	Experimental, I3	-2.3	2.07	-1.11	1.000
	Active, Control, I3	10.9	3.18	3.43	0.084
Active, Control, I2	Experimental, I3	-11.3	3.18	-3.55	0.063
	Active, Control, I3	1.9	2.07	0.91	1.000
Experimental, I3	Active, Control, I3	13.2	2.88	4.58	0.006

Note: I; Intervention.

Table 8. Medical Outcomes Study Questionnaire Short Form 36 Health Survey – *t*-test – Improvement difference between groups.

	Experimental Group	**Active Control Group**
Mean	6.8	0.98
Observations	10	10
df	18	
t statistic	7.03	
p - value	< 0.05	

Modified Fatigue Impact Scale

Table 9. Modified Fatigue Impact Scale – Within Subjects Effects.

Cases	F	*p*
Study Phases	11.18	< 0.001
Study Phases ✻ Group	13.88	< 0.001

Note: ✻; Interaction between the two variables.

Table 10. Modified Fatigue Impact Scale – Post Hoc Comparisons – Study Phases.

		Mean Difference	SE	t	p_{bonf}
Baseline	I1	3.700	1.143	3.238	0.027
	I2	5.250	1.343	3.909	0.006
	I3	6.200	1.606	3.860	0.007
I1	I2	1.550	0.593	2.615	0.105
	I3	2.500	1.170	2.137	0.280
I2	I3	0.950	0.754	1.259	1.000

Note. I; Intervention. Results are averaged over the levels of: Group.

Table 11. Modified Fatigue Impact Scale – Post Hoc Comparisons – Group ✻ Study Phases.

		Mean Difference	SE	t	p_{bonf}
Experimental, Baseline	Active, Control, Baseline	-7.4	7.88	-0.9	1.000
	Experimental, I1	7.7	1.61	4.76	0.004

	Active, Control, I1	-7.7	7.78	-0.1	1.000
	Experimental, I2	11.1	1.89	5.84	< 0.001
	Active, Control, I2	-8	7.91	-1	1.000
	Experimental, I3	13.1	2.27	5.76	< 0.001
	Active, Control, I3	-8.1	8.23	-0.1	1.000
Active, Control, Baseline	Experimental, I1	15.1	7.78	1.94	1.000
	Active, Control, I1	-0.3	1.61	-0.2	1.000
	Experimental, I2	18.5	7.91	2.33	0.873
	Active, Control, I2	-0.6	1.89	-0.3	1.000
	Experimental, I3	20.5	8.23	2.49	0.637
	Active, Control, I3	-0.7	2.27	-0.3	1.000
Experimental, I1	Active, Control, I1	-15.4	7.68	-2	1.000
		3.4	0.83	4.05	0.021
	Active, Control, I2	-15.7	7.81	-2	1.000
	Experimental, I3	5.4	1.65	3.26	0.121
	Active, Control, I3	-15.8	8.13	-1.9	1.000
Active, Control, I1	Experimental, I2	18.8	7.81	2.4	0.759
	Active, Control, I2	-0.3	0.83	-0.3	1.000
	Experimental, I3	20.8	8.13	2.55	0.555
	Active, Control, I3	-0.4	1.65	-0.2	1.000
Experimental, I2	Active, Control, I2	-19.1	7.94	-2.4	0.761
	Experimental, I3	2	1.06	1.87	1.000
	Active, Control, I3	-19.2	8.26	-2.3	0.896
Active, Control, I2	Experimental, I3	21.1	8.26	2.55	0.558

	Active, Control, I3	-0.1	1.06	-0.1	1.000
Experimental, I3	Active, Control, I3	-21.2	8.56	-2.4	0.658

Note: I; Intervention.

Table 12. Modified Fatigue Impact Scale – *t*-test – Improvement difference between groups.

	Experimental Group	**Active Control Group**
Mean	10.6	0.29
Observations	10	10
df	18	
t statistic	4.1	
p - value	< 0.05	

Purdue Pegboard Test

Table 13. Purdue Pegboard Test – Within Subjects Effects.

Cases	**F**	***p***
Study Phases	45.95	< 0.001
Study Phases ✻ Group	43.53	< 0.001
Subtests	100.23	< 0.001
Subtests ✻ Group	4.51	0.007
Subtests ✻ Subtests ✻Group	6.6	< 0.001

Note: ✻; Interaction between the two variables.

Table 14. Purdue Pegboard test – Post Hoc Comparisons – Study Phases.

		Mean Difference	SE	t	p_{bonf}
Baseline	Intervention	-1.25	0.18	-6.77	< 0.001

Note: Results are averaged over the levels of: Group, Subtests, Assessment Points.

Table 15. Purdue Pegboard test – Post Hoc Comparisons – Subtests.

		Mean Difference	SE	t	p_{bonf}
Unimanual Dominant	Unimanual Non-Dominant	1.93	0.23	8.40	< 0.001
	Bimanual	2.43	0.29	8.35	< 0.001
	Assembly	-5.48	0.62	-8.71	< 0.001
Unimanual Non-Dominant	Bimanual	0.5	0.23	2.15	0.270
	Assembly	-7.41	0.63	-11.62	< 0.001
Bimanual	Assembly	-7.91	0.76	-10.41	< 0.001

Note: Results are averaged over the levels of: Group, Assessment Points, Study Phases.

Table 16. Purdue Pegboard test – Post Hoc Comparisons – Group ✻ Study Phases ✻ Subtests.

		Mean Difference	SE	t	p_{bonf}
Experimental, Baseline, Unimanual Dominant	Active, Control, Baseline, Unimanual Dominant	1.5	0.94	1.58	1.000

	Experimental, Intervention, Unimanual Dominant	-2.03	0.32	-6.25	< 0.001
	Active, Control, Intervention, Unimanual Dominant	1.66	0.9	1.84	1.000
	Experimental, Baseline, Unimanual Non-Dominant	1.93	0.35	5.39	0.005
	Active, Control, Baseline, Unimanual Non-Dominant	3.06	0.96	3.17	0.623
	Experimental, Intervention, Unimanual Non-Dominant	0.43	0.5	0.86	1.000
	Active, Control, Intervention, Unimanual Non-Dominant	3.43	0.86	3.95	0.111
	Experimental, Baseline, Bimanual	2.83	0.47	5.92	0.002
	Active, Control, Baseline, Bimanual	3.63	0.82	4.42	0.04

	Experimental, Intervention, Bimanual	1.1	0.51	2.13	1.000
	Active, Control, Intervention, Bimanual	3.3	0.82	4.02	0.096
	Experimental, Baseline, Assembly	-5.4	0.838	-6.44	< 0.001
	Active, Control, Baseline, Assembly	-2.53	1.44	-1.75	1.000
	Experimental, Intervention, Assembly	-10	0.94	-10.6	< 0.001
	Active, Control, Intervention, Assembly	-2.86	1.54	-1.85	1.000
Active, Control, Baseline, Unimanual Dominant	Experimental, Intervention, Unimanual Dominant	-3.53	0.9	-3.91	0.121
	Active, Control, Intervention, Unimanual Dominant	0.16	0.32	0.51	1.000
	Experimental, Baseline, Unimanual Non-Dominant	0.43	0.96	0.44	1.000

	Active, Control, Baseline, Unimanual Non-Dominant	1.56	0.35	4.37	0.044
	Experimental, Intervention, Unimanual Non-Dominant	-1.06	0.86	-1.22	1.000
	Active, Control, Intervention, Unimanual Non-Dominant	1.93	0.5	3.84	0.143
	Experimental, Baseline, Bimanual	1.33	0.82	1.62	1.000
	Active, Control, Baseline, Bimanual	2.13	0.47	4.45	0.036
	Experimental, Intervention, Bimanual	-0.4	0.82	-0.48	1.000
	Active, Control, Intervention, Bimanual	1.8	0.51	3.5	0.307
	Experimental, Baseline, Assembly	-6.9	1.44	-4.76	0.018
	Active, Control, Baseline, Assembly	-4.03	0.83	-4.81	0.017
	Experimental, Intervention, Assembly	-11.5	1.54	-7.45	< 0.001

	Active, Control, Intervention, Assembly	-4.36	0.94	-4.63	0.025
Experimental, Intervention, Unimanual Dominant	Active, Control, Intervention, Unimanual Dominant	3.7	0.85	4.33	0.049
	Experimental, Baseline, Unimanual Non-Dominant	3.96	0.46	8.60	< 0.001
	Active, Control, Baseline, Unimanual Non-Dominant	5.1	0.92	5.54	0.004
	Experimental, Intervention, Unimanual Non-Dominant	2.46	0.39	6.24	< 0.001
	Active, Control, Intervention, Unimanual Non-Dominant	5.46	0.81	6.67	< 0.001
	Experimental, Baseline, Bimanual	4.86	0.46	10.54	< 0.001
	Active, Control, Baseline, Bimanual	5.66	0.77	7.35	< 0.001
	Experimental, Intervention, Bimanual	3.13	0.42	7.44	< 0.001

	Active, Control, Intervention, Bimanual	5.33	0.77	6.93	< 0.001
	Experimental, Baseline, Assembly	-3.37	0.92	-3.63	0.226
	Active, Control, Baseline, Assembly	-0.5	1.41	-0.35	1.000
	Experimental, Intervention, Assembly	-7.96	1.01	-7.83	< 0.001
	Active, Control, Intervention, Assembly	-0.83	1.51	-0.55	1.000
Active, Control, Intervention, Unimanual Dominant	Experimental, Baseline, Unimanual Non-Dominant	0.26	0.92	0.29	1.000
	Active, Control, Baseline, Unimanual Non-Dominant	1.4	0.46	3.03	0.853
	Experimental, Intervention, Unimanual Non-Dominant	-1.23	0.82	-1.5	1.000
	Active, Control, Intervention, Unimanual Non-Dominant	1.76	0.39	4.47	0.035

	Experimental, Baseline, Bimanual	1.16	0.77	1.51	1.000
	Active, Control, Baseline, Bimanual	1.96	0.46	4.26	0.057
	Experimental, Intervention, Bimanual	-0.56	0.77	-0.73	1.000
	Active, Control, Intervention, Bimanual	1.63	0.42	3.88	0.132
	Experimental, Baseline, Assembly	-7.067	1.418	-4.98	0.012
	Active, Control, Baseline, Assembly	-4.2	0.92	-4.54	0.031
	Experimental, Intervention, Assembly	-11.66	1.51	-7.7	< 0.001
	Active, Control, Intervention, Assembly	-4.53	1.01	-4.45	0.036
Experimental, Baseline, Unimanual Non-Dominant	Active, Control, Baseline, Unimanual Non-Dominant	1.13	0.98	1.15	1.000
	Experimental, Intervention, Unimanual Non-Dominant	-1.5	0.47	-3.19	0.604

	Active, Control, Intervention, Unimanual Non-Dominant	1.5	0.88	1.69	1.000
	Experimental, Baseline, Bimanual	0.9	0.43	2.09	1.000
	Active, Control, Baseline, Bimanual	1.7	0.84	2.01	1.000
	Experimental, Intervention, Bimanual	-0.83	0.54	-1.54	1.000
	Active, Control, Intervention, Bimanual	1.36	0.84	1.62	1.000
	Experimental, Baseline, Assembly	-7.33	0.87	-8.41	< 0.001
	Active, Control, Baseline, Assembly	-4.46	1.46	-3.06	0.805
	Experimental, Intervention, Assembly	-11.93	0.9	-13.24	< 0.001
	Active, Control, Intervention, Assembly	-4.8	1.55	-3.09	0.758
Active, Control, Baseline, Unimanual Non-Dominant	Experimental, Intervention, Unimanual Non-Dominant	-2.63	0.88	-2.96	0.991

	Active, Control, Intervention, Unimanual Non-Dominant	0.36	0.47	0.78	1.000
	Experimental, Baseline, Bimanual	-0.23	0.84	-0.27	1.000
	Active, Control, Baseline, Bimanual	0.56	0.43	1.31	1.000
	Experimental, Intervention, Bimanual	-1.96	0.84	-2.33	1.000
	Active, Control, Intervention, Bimanual	0.23	0.54	0.43	1.000
	Experimental, Baseline, Assembly	-8.46	1.46	-5.8	0.002
	Active, Control, Baseline, Assembly	-5.6	0.87	-6.42	< 0.001
	Experimental, Intervention, Assembly	-13.06	1.55	-8.41	< 0.001
	Active, Control, Intervention, Assembly	-5.93	0.9	-6.58	< 0.001
Experimental, Intervention, Unimanual Non-Dominant	Active, Control, Intervention , Unimanual Non-Dominant	3	0.78	3.83	0.144

	Experimental, Baseline, Bimanual	2.4	0.38	6.18	< 0.001
	Active, Control, Baseline, Bimanual	3.2	0.73	4.38	0.043
	Experimental, Intervention, Bimanual	0.66	0.35	1.88	1.000
	Active, Control, Intervention, Bimanual	2.86	0.73	3.93	0.117
	Experimental, Baseline, Assembly	-5.83	1.02	-5.71	0.002
	Active, Control, Baseline, Assembly	-2.96	1.39	-2.12	1.000
	Experimental, Intervention, Assembly	-10.43	1.03	-10.07	< 0.001
	Active, Control, Intervention, Assembly	-3.3	1.49	-2.2	1.000
Active, Control, Intervention, Unimanual Non-Dominant	Experimental, Baseline, Bimanual	-0.6	0.73	-0.82	1.000
	Active, Control, Baseline, Bimanual	0.2	0.38	0.51	1.000
	Experimental, Intervention, Bimanual	-2.33	0.73	-3.2	0.594

	Active, Control, Intervention, Bimanual	-0.13	0.35	-0.37	1.000
	Experimental, Baseline, Assembly	-8.83	1.39	-6.32	< 0.001
	Active, Control, Baseline, Assembly	-5.96	1.02	-5.84	0.002
	Experimental, Intervention, Assembly	-13.43	1.49	-8.98	< 0.001
	Active, Control, Intervention, Assembly	-6.3	1.03	-6.08	0.001
Experimental, Baseline, Bimanual	Active, Control, Baseline, Bimanual	0.8	0.67	1.18	1.000
	Experimental, Intervention, Bimanual	-1.73	0.31	-5.48	0.004
	Active, Control, Intervention, Bimanual	0.46	0.67	0.69	1.000
	Experimental, Baseline, Assembly	-8.23	1.06	-7.72	< 0.001
	Active, Control, Baseline, Assembly	-5.36	1.37	-3.92	0.120
	Experimental, Intervention, Assembly	-12.83	1.13	-11.3	< 0.001

	Active, Control, Intervention, Assembly	-5.7	1.47	-3.88	0.132
Active, Control, Baseline, Bimanual	Experimental, Intervention, Bimanual	-2.53	0.67	-3.76	0.172
	Active, Control, Intervention, Bimanual	-0.33	0.31	-1.05	1.000
	Experimental, Baseline, Assembly	-9.03	1.37	-6.6	< 0.001
	Active, Control, Baseline, Assembly	-6.16	1.06	-5.78	0.002
	Experimental, Intervention, Assembly	-13.63	1.47	-9.28	< 0.001
	Active, Control, Intervention, Assembly	-6.5	1.13	-5.75	0.002
Experimental, Intervention, Bimanual	Active, Control, Intervention, Bimanual	2.2	0.67	3.27	0.507
	Experimental, Baseline, Assembly	-6.5	1.1	-5.91	0.002
	Active, Control, Baseline, Assembly	-3.63	1.36	-2.65	1.000
	Experimental, Intervention, Assembly	-11.1	1.14	-9.68	< 0.001

	Active, Control, Intervention, Assembly	-3.96	1.47	-2.7	1.000
Active, Control, Intervention, Bimanual	Experimental, Baseline, Assembly	-8.7	1.36	-6.36	< 0.001
	Active, Control, Baseline, Assembly	-5.83	1.1	-5.3	0.006
	Experimental, Intervention, Assembly	-13.3	1.47	-9.05	< 0.001
	Active, Control, Intervention, Assembly	-6.16	1.14	-5.38	0.005
Experimental, Baseline, Assembly	Active, Control, Baseline, Assembly	2.86	1.81	1.58	1.000
	Experimental, Intervention, Assembly	-4.6	0.45	-10	< 0.001
	Active, Control, Intervention, Assembly	2.53	1.89	1.34	1.000
Active, Control, Baseline, Assembly	Experimental, Intervention, Assembly	-7.46	1.89	-3.94	0.113
	Active, Control, Intervention, Assembly	-0.33	0.45	-0.72	1.000

Experimental, Intervention, Assembly	Active, Control, Intervention, Assembly	7.13	1.96	3.63	0.230

Note: Results are averaged over the levels of: Assessment Points.

Table 17. Purdue Pegboard Subtests improvement – Within Subjects Effects.

Cases	F	*p*
Purdue Pegboard Subtests	11.51	< 0.001
Purdue Pegboard Subtests ✻ Group	6.6	< 0.001

Table 18. Purdue Pegboard Subtests improvement – Post Hoc Comparisons – Group ✻ Subtests.

		Mean Difference	SE	t	p_{bonf}
Experimental, Unimanual Dominant	Active, Control, Unimanual Dominant	2.2	0.46	4.7	0.004
	Experimental, Unimanual Non-Dominant	0.53	0.38	1.4	1.000
	Active, Control, Unimanual Non-Dominant	2.4	0.57	4.2	0.015
	Experimental, Bimanual	0.3	0.36	0.82	1.000
	Active, Control, Bimanual	1.700	0.453	3.75	0.041

	Experimental, Assembly	-2.567	0.554	-4.6	0.006
	Active, Control, Assembly	1.700	0.562	3.02	0.203
Active, Control, Unimanual Dominant	Experimental, Unimanual Non-Dominant	-1.66	0.57	-2.9	0.258
	Active, Control, Unimanual Non-Dominant	0.2	0.38	0.52	1.000
	Experimental, Bimanual	-1.9	0.45	-4.2	0.015
	Active, Control, Bimanual	-0.5	0.36	-1.3	1.000
	Experimental, Assembly	-4.76	0.562	-8.4	< 0.001
	Active, Control, Assembly	-0.5	0.55	-0.9	1.000
Experimental, Unimanual Non-Dominant	Active, Control, Unimanual Non-Dominant	1.86	0.66	2.81	0.324
	Experimental, Bimanual	-0.23	0.43	-0.5	1.000
	Active, Control, Bimanual	1.16	0.566	2.06	1.000
	Experimental, Assembly	-3.1	0.63	-4.8	0.004

	Active, Control, Assembly	1.16	0.656	1.77	1.000
Active, Control, Unimanual Non-Dominant	Experimental, Bimanual	-2.1	0.56	-3.7	0.045
	Active, Control, Bimanual	-0.7	0.43	-1.6	1.000
	Experimental, Assembly	-4.96	0.65	-7.5	< 0.001
	Active, Control, Assembly	-0.7	0.63	-1	1.000
Experimental, Bimanual	Active, Control, Bimanual	1.4	0.44	3.13	0.160
	Experimental, Assembly	-2.86	0.52	-5.4	< 0.001
	Active, Control, Assembly	1.4	0.55	2.51	0.603
Active, Control, Bimanual	Experimental, Assembly	-4.26	0.55	-7.6	< 0.001
	Active, Control, Assembly	$1.\times10^{-10}$	0.52	2×10^{-10}	1.000
Experimental, Assembly	Active, Control, Assembly	4.26	0.64	6.58	< 0.001

Timed 25-Foot Walk Test

Table 19. Timed 25-Foot Walk Test – Within Subjects Effects.

Cases	F	*p*
Study Phases	69.87	< 0.001
Study Phases ✻ Group	76.68	< 0.001

Note: ✻; Interaction between the two variables.

Table 20. Timed 25-Foot Walk Test – Post Hoc Comparisons – Study phases.

		Mean Difference	SE	t	p_{bonf}
Baseline	Intervention	1.08	0.13	8.36	< 0.001

Note: Results are averaged over the levels of: Group, Assessments Points.

Table 21. Timed 25-Foot Walk Test – Post Hoc Comparisons – Group ✻ Study Phases.

		Mean Difference	SE	t	p_{bonf}
Experimental, Baseline	Active, Control, Baseline	-0.48	1.12	-0.42	1.000
	Experimental, Intervention	2.22	0.18	12.1	< 0.001
	Active, Control, Intervention	-0.53	1.11	-0.4	1.000
Active, Control, Baseline	Experimental, Intervention	2.7	1.11	2.42	0.156
	Active, Control, Intervention	-0.05	0.18	-0.2	1.000
Experimental, Intervention	Active, Control, Intervention	-2.75	1.10	-2.4	0.135

Note: Results are averaged over the levels of: Assessments Points.

Table 22. Timed 25-Foot Walk Test – *t*-test – Improvement difference between groups.

	Experimental Group	**Active Control Group**
Mean	2.2	-0.05
Observations	10	10
df	18	
t statistic	8.5	
p - value	< 0.05	

Six Spot Step Test

Table 23. Six Spot Step Test – Within Subjects Effects.

Cases	**F**	***p***
Study Phases	15.43	< 0.001
Study Phases ✻ Group	19.67	< 0.001

Note: ✻; Interaction between the two variables.

Table 24. Six Spot Step Test – Post Hoc Comparisons – Study – Study phases.

		Mean Difference	**SE**	**t**	p_{bonf}
Baseline	Intervention	1.09	0.28	3.93	< 0.001

Note: Results are averaged over the levels of: Group, Assessments Points.

Table 25. Six Spot Step Test – Post Hoc Comparisons - Group ✻ Study Phases.

		Mean Difference	SE	t	p_{bonf}
Experimental, Baseline	Active, Control, Baseline	-3.05	3.83	-0.8	1.000
	Experimental, Intervention	2.33	0.39	5.91	< 0.001
	Active, Control, Intervention	-3.19	3.69	-0.8	1.000
Active, Control, Baseline	Experimental, Intervention	5.39	3.69	1.46	0.967
	Active, Control, Intervention	-0.14	0.39	-0.3	1.000
Experimental, Intervention	Active, Control, Intervention	-5.53	3.54	-1.5	0.814

Note: Results are averaged over the levels of: Assessment Points.

Table 26. Six Spot Step Test – *t*-test – Improvement difference between groups.

	Experimental Group	Active Control Group
Mean	2.3	-0.1
Observations	10	10
df	9	
t statistic	4	
p - value	< 0.05	