Aus der Klinik für Neurologie
der Heinrich-Heine-Universität Düsseldorf
Direktor: Univ.-Prof. Dr. med. Dr. rer. nat. Sven Meuth

The Evaluation of Quadripulse Stimulation-Induced LTP-Like Plasticity as a Neurophysiological Biomarker in Different Subtypes of Multiple Sclerosis

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
der Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Arved-Sebastian Stucke

2025

Als Inauguraldissertation gedruckt mit Genehmigung der
Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

gez.:
Dekan: Prof. Dr. med. Nikolaj Klöcker
Erstgutachter: Prof. Dr. med. Philipp Albrecht
Zweitgutachter: Prof. Dr. med. Ulrich Germing

To my parents, for their endless support and encouragement.

Teile dieser Arbeit wurden veröffentlicht:

BALLOFF, C., ALBRECHT, P., STUCKE, A. S., SCALA, L., NOVELLO, S., HARTMANN, C. J., MEUTH, S. G., SCHNITZLER, A., PENNER, I. K. & GROISS, S. J. 2023. The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis. *Front Neurol,* 14**,** 1266225.

BALLOFF, C., NOVELLO, S., STUCKE, A. S., JANSSEN, L. K., HEINEN, E., HARTMANN, C. J., MEUTH, S. G., SCHNITZLER, A., PENNER, I. K., ALBRECHT, P. & GROISS, S. J. 2023. Long-term potentiation-like plasticity is retained during relapse in patients with Multiple Sclerosis. *Clin Neurophysiol,* 155**,** 76-85.

Summary (English)

Until now, biomarkers of multiple sclerosis (MS) fail to explain the clinico-radiological paradox (CRP): the phenomenon that some people with MS show little or no symptoms despite extensive damage to the central nervous system (CNS), while others, who only show minimal CNS damage, exhibit extensive clinical disability. Yet, differences in neural plasticity could help to explain this paradox. Neural plasticity can possibly be quantified by means of long-term potentiation (LTP)-like plasticity. In that regard, previous research showed that quadripulse stimulation (QPS), a repetitive transcranial magnetic stimulation (rTMS) protocol, induces LTP-like plasticity with little fluctuation in healthy individuals. Hence, the aim of this thesis was to investigate, whether the extent of QPS-induced LTP-like plasticity differs between progressive subtypes of MS (PMS), stable relapsing-remitting MS (RRMS), and healthy individuals and can thus, serve as a diagnostic und prognostic biomarker of MS, which is not conflicted by the CRP.

Therefore, a study was conducted in which, $n = 34$ participants with PMS were matched in age, sex and education with each $n = 30$ participants with RRMS and $n = 30$ healthy controls (HC). LTP-like plasticity was induced by applying QPS at a frequency of 5 Hz for 30 minutes over the left primary motor cortex and was measured at one pre-QPS and six post-QPS assessments by means of the amplitude of 12 rTMS triggered motor evoked potentials (MEP) of the right first dorsal interosseus muscle. To determine differences in the increment of LTP-like plasticity between the three groups, the highest mean post-QPS MEP amplitude was compared with the mean pre-QPS MEP amplitude in a multilevel mixed model. The analysis showed a significant increase in MEP amplitude from pre-QPS to post-QPS assessment across all groups, $p < .001$. However, this increment did not differ significantly between groups, $p = .497$. Yet, a non-significant trend could be observed suggesting that individuals with PMS have a lower MEP increment than individuals with stable RRMS and HC. Moreover, post-hoc analysis revealed that across groups, participants with longer MEP latencies had significantly lower increments in MEP amplitude, $p < .001$.

This thesis provides evidence that QPS is a powerful rTMS paradigm which is able to induce LTP-like plasticity not only in healthy individuals, but also in individuals with different subtypes of MS. However, there seems to be no difference in LTP-like plasticity between these groups, which in light of the observed trends could be interpreted as a lack of statistical power due to methodological limitations in the rTMS application. In addition, the degree of pyramidal tract integrity as represented by MEP latency, seems to account for differences in LTP-like plasticity. Thus, future studies should address these methodological limitations, for example through an artificial intelligence-based personalized rTMS approach and the application of a higher number of rTMS stimuli. Moreover, alongside MS subtype, the role of pyramidal tract integrity should be investigated to determine the diagnostic and prognostic value of LTP-like plasticity for MS progression, relapse and recovery.

Summary (German)

Bisher können die verfügbaren Biomarker der Multiplen Sklerose (MS) das klinisch-radiologische Paradox (KRP) nicht erklären: Das Phänomen, dass einige Personen mit MS trotz ausgeprägter Schädigung des zentralen Nervensystems (ZNS) nur minimale oder keine Symptome aufweisen, während andere, die nur geringgradige ZNS-Schädigungen zeigen, eine erhebliche klinische Behinderung aufweisen. Unterschiede in der neuronalen Plastizität könnten dieses Paradox jedoch erklären. Die neuronale Plastizität lässt sich dabei möglicherweise elektrophysiologisch als Langzeitpotenzierung (LTP) quantifizieren. Diesbezüglich zeigten frühere Studien, dass die Quadripulse Stimulation (QPS), ein Protokoll der repetitiven transkraniellen Magnetstimulation (rTMS), LTP-Plastizität mit nur geringen Fluktuationen bei Gesunden induziert. Ziel dieser Doktorarbeit war es deshalb zu untersuchen, ob sich das Ausmaß der LTP-Plastizität zwischen progressiven Subtypen der MS (PMS), stabiler schubförmig remittierender MS (RRMS) sowie gesunden Personen unterscheidet und damit als diagnostischer und prognostischer Biomarker der MS dienen kann, welcher nicht im Widerspruch zum KRP steht.

Hierfür wurde eine Studie durchgeführt in welcher n = 34 Probanden mit PMS in Alter, Geschlecht und Bildungsgrad mit jeweils n = 30 Probanden mit stabiler RRMS und n = 30 gesunden Kontrollen (HC) gematcht wurden. Die LTP-Plastizität wurde durch 30-minütige QPS des linken primären Motorcortex mit einer Frequenz von 5 Hz induziert und mittels einer Prä-QPS und sechs Post-QPS rTMS Messungen anhand der Amplitude von je 12 motorisch evozierten Potenzialen (MEP) des rechten Musculus interosseus dorsalis I gemessen. Um Unterschiede im Anstieg der LTP-Plastizität zu bestimmen, wurde die höchste gemittelte Post-QPS MEP Amplitude mit der gemittelten Prä-QPS MEP Amplitude der drei Gruppen in einer Mehrebenenanalyse verglichen. Die Analyse zeigte einen signifikanten, gruppen-übergreifenden Anstieg der MEP-Amplitude von der Prä- zur Post-QPS Messung, $p < .001$. Dieser Anstieg wies jedoch zwischen den Gruppen keinen signifikanten Unterschied auf, $p = .497$. Allerdings konnte ein nicht signifikanter Trend beobachtet werden, der daraufhin deutet, dass Probanden mit PMS einen geringeren MEP-Anstieg aufweisen, als Probanden mit stabiler RRMS und HC. Zudem zeigten Post-hoc-Tests, dass Probanden mit längeren MEP-Latenzzeiten gruppenübergreifend signifikant geringere Anstiege der MEP-Amplitude aufwiesen, $p < .001$.

Die im Rahmen dieser Doktorarbeit durchgeführten Untersuchungen zeigten, dass QPS ein starkes rTMS-Paradigma ist, das nicht nur bei gesunden Personen, sondern auch bei Personen mit verschiedenen MS-Subtypen zuverlässig LTP-Plastizität induziert. Jedoch scheint es zwischen diesen Gruppen keinen Unterschied in der LTP-Plastizität zu geben, was hinsichtlich der beobachteten Trends als ein Mangel an statistischer Power aufgrund von methodologischen Limitierungen in der rTMS-Applikation interpretiert werden könnte. Ferner scheint insbesondere der Grad der Pyramidenbahnintegrität, repräsentiert durch die MEP-Latenz, für LTP-Plastizitäts-unterschiede verantwortlich zu sein. Zukünftige Studien, sollten diese methodologischen Limitierungen zum Beispiel durch einen auf künstlicher Intelligenz basierenden, personalisierten rTMS-Ansatz oder eine Erhöhung der rTMS Stimulus Anzahl adressieren. Darüber hinaus sollten neben den MS-Subtypen die Rolle der Pyramidenbahnintegrität untersucht werden, um den diagnostischen und prognostischen Wert der LTP-Plastizität für das Fortschreiten, den Rückfall und die Genesung der MS zu bestimmen.

List of Abbreviations

AIC	Akaike's Information Criterion
AMPA	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid
AMT	Active Motor Threshold
ANOVA	Analysis of Variance
BDNF	Brain-Derived Neurotropic Factor
BVMT-R	Brief Visuospatial Memory Test - Revised
CERAD	Consortium to Establish a Registry for Alzheimer's Disease Neuropsychological Test Battery
CIS	Clinical Isolated Syndrome
cm	Centimeter
CNS	Central Nervous System
COVID-19	Corona Virus Disease 2019
CRP	Clinico-Radiological Paradox
CSF	Corticospinal Fluid
cTBS	Continuous Theta Burst Stimulation
EBV	Epstein-Barr Virus
EDSS	Expanded Disability Status Scale
EEG	Electroencephalography
EMG	Electromyography
FDA	Food and Drug Administration
FDI	First Dorsal Interosseus Muscle
FSMC	Fatigue Scale for Motor & Cognitive Functions
H	Hypothesis
HADS	Hospital Anxiety & Depression Scale
HC	Healthy Control
HLA II	Human Leukocyte Antigen Class II
Hz	Hertz
IBI	Interburst Interval
ISI	Interstimulus Interval
iTBS	Intermittent Theta Burst Stimulation
kHz	Kilohertz
LTD	Long Term Depression
LTP	Long Term Potentiation
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
mm	Millimeter
MRI	Magnetic Resonance Imaging
ms	Millisecond
MS	Multiple Sclerosis
μV	Microvolt
mV	Millivolt
NIH	National Institutes of Health
NMDA	N-Methyl-D-Aspartate
NHPT	The 9-Hole-Peg Test

PAS	Paired Associative Stimulation
PDGF	Platelet-Derived Growth Factor
PMS	Progressive Multiple Sclerosis
PPMS	Primary Progressive Multiple Sclerosis
QPS	Quadripulse Stimulation
RMT	Resting Motor Threshold
RRMS	Relapsing Remitting Multiple Sclerosis
rTMS	Repetitive Transcranial Magnetic Stimulation
s	Second
SDMT	Symbol Digit Modalities Test
SPMS	Secondary Progressive Multiple Sclerosis
T25FW	The 25 Foot Walk
TBS	Theta Burst Stimulation
TMS	Transcranial Magnetic Stimulation
TNF-α	Tumor Necrosis Factor-α
UHD	University Hospital Düsseldorf

Table of Contents

1. Introduction

Multiple sclerosis (MS) is a debilitating autoimmune demyelinating disease of the central nervous system (CNS), characterized by neuroinflammatory and neurodegenerative processes (Kunze et al., 2023, Coupe et al., 2023, Cheriyan et al., 2012). As the most frequent demyelinating disease, it affects about 108 individuals per 100000 people in Europe and about 2.8 million worldwide (Walton et al., 2020, Leray et al., 2016). The first manifestation occurs in young adulthood and life expectancy is reduced by about 6-14 years (Leray et al., 2015, Leray et al., 2016). Due to this high prevalence, early age of onset and progressive disabling disease course, the damage for society and economy is severe (Romero-Pinel et al., 2022).

Progression of MS occurs either through reoccurring (relapse-associated), inflammatory, grey and white matter lesions or relapse-independent neurodegenerative processes (Lublin et al., 2022). Yet, the number of grey and white matter lesions depicted in magnetic resonance imaging (MRI) poorly predict clinical symptoms (Uher et al., 2018, Dunschede et al., 2023). This phenomenon is referred to as the clinico-radiological paradox (CRP) (Mollison et al., 2017, Barkhof, 2002). The resolvement of the CRP is important, as MRI of the brain and spinal cord are to date, the most prominent diagnostic and prognostic biomarkers of MS (Thompson et al., 2018, Housley et al., 2015).

The concept of neuroplasticity could pose a solution to the CRP (Tremblay et al., 2023, Stampanoni Bassi et al., 2019c, Mori et al., 2014a). Through synaptic strengthening and weakening by means of long-term-potentiation (LTP) and long-term depression (LTD), neuroplasticity could compensate for functional and structural damages of the CNS (Stampanoni Bassi et al., 2019c, Stampanoni Bassi et al., 2019a, Massey and Bashir, 2007). Conversely, when these compensatory mechanisms are depleted, CNS damage may express itself in the form of progressing clinical symptoms (Stampanoni Bassi et al., 2019b, Mori et al., 2013). Thus, neuroplasticity may be a suitable biomarker of MS (Snow et al., 2019).

Using non-invasive repetitive transcranial magnetic stimulation (rTMS), LTP- and LTD-like plasticity can be assessed on a cortical level: cortical plasticity (Klomjai et al., 2015, Barker et al., 1985). In the last decades, different rTMS protocols were designed that either induce LTP- or LTD-like cortical plasticity (Klomjai et al., 2015). However, due to high fluctuations in their ability to induce cortical plasticity, it cannot yet serve as a biomarker for MS (Snow et al., 2019, Corp et al., 2020). A new rTMS paradigm, able to induce LTP- and LTD-like cortical plasticity with only little fluctuations is quadripulse stimulation (QPS) (Tiksnadi et al., 2020, Nakamura et al., 2016). Consequently, it needs to be investigated whether the extent of QPS-induced cortical plasticity can serve as a Biomarker of MS.

This thesis focused on investigating, whether the extent of QPS-induced rapid onset LTP-like plasticity differs between progressive subtypes of MS (PMS), stable relapsing-remitting MS (RRMS), and healthy individuals and can thus, serve as a diagnostic und prognostic biomarker of MS. Accordingly, the following chapters provide a detailed overview of MS by means of its definition, epidemiology, classification, etiology and pathophysiology, as well as the current state of its diagnostic and prognostic biomarkers. After that, the concept and role of cortical plasticity, specifically LTP-like plasticity, will be introduced and LTP-like plasticity induction through rTMS will be explained. Thereafter, the current state of rTMS research in MS will be reviewed, leading to the abovementioned aim of this thesis. Subsequently, the study design will be outlined and the results be presented. In the end, the results will be summarized and discussed in light of current research in the field.

1.1 Multiple Sclerosis

1.1.1 Definition and Epidemiology

MS is the most frequent autoimmune demyelinating disease of the CNS that affects about 2.8 million worldwide (Walton et al., 2020). Due to the heterogeneity in its clinical manifestation and unpredictability in its progression, MS is often called the disease with 1000 faces (Gross et al., 2019, Engelhardt et al., 2022). Regarding the prevalence and incidence, people who are living farther from the equator are more often affected than those, who are living closer to it (Walton et al., 2020, Sabel et al., 2021). As with other autoimmune diseases, women are more often affected than men (Koch-Henriksen and Sorensen, 2010), with a risk of about 0.5 percent in females and 0.3 percent in males in the general population (Nielsen et al., 2005). Compared to this general risk, siblings of afflicted individuals have a 3 – 14 percent risk and monozygotic twins have a risk of about 30 percent to develop MS (Kuusisto et al., 2008, Hansen et al., 2005). The mean age of onset is about 30 years and a typical first manifestation is the opticus neuritis (Koch-Henriksen and Sorensen, 2010, Dobson and Giovannoni, 2019). Other symptoms that may appear at onset or during the course of disease are paraesthesia, paresis, cerebellar and vestibular symptoms, vegetative symptoms, fatigue, cognitive dysfunction and psychiatric symptoms such as depression (Noseworthy et al., 2000, Doshi and Chataway, 2017).

The diagnosis of MS, in this study, is based on the 2017 revised McDonald criteria, which regard the clinical presentation, the dissemination of MRI grey and white matter lesions in space (e.g. cortical, juxtacortical, cerebellar, periventricular, subtentorial, infratentorial or spinal cord lesions) and time (e.g. gadolinium enhanced lesions next to unenhanced lesions or additional lesions at different times of MRI assessment), as well as the presence of oligoclonal bands in the corticospinal fluid (CSF) (Thompson et al., 2018). A revision of the diagnostic criteria is currently underway and was presented at the European Committee for Treatment and Research in Multiple Sclerosis Conference in 2024. Concerning the clinical presentation, MS usually manifests itself in an attack, which is defined as the symptomatic aggravation lasting for at least 24 hours (Hauser and Cree, 2020). This attack is followed by a period of partial or full recovery before a further attack occurs (Dobson and Giovannoni, 2019). By definition further attacks have to be separated from a previous attack by at least 30 days to be counted as such (Hauser and Cree, 2020). Nevertheless, apart from the course of recuring attacks, progressive disease courses at the time of onset or after an initial period of attacks are also common, resulting in the current classification of MS (Lublin et al., 2014).

1.1.2 Classification

According to clinical consensus, there are four different MS phenotypes: RRMS, primary progressive multiple sclerosis (PPMS), secondary progressive multiple sclerosis (SPMS) and the clinical isolated syndrome (CIS) (Lublin et al., 2014, Lublin et al., 2020). RRMS is the most frequent type of MS, accounting for about 70 to 85 percent of the cases (Noseworthy et al., 2000, Hunter, 2016, Broos et al., 2024). RRMS is dominated by inflammatory processes and characterized by attacks of neurologic symptoms that may either be new or regressing (Papiri et al., 2023, Lublin et al., 2014, Lublin and Reingold, 1996). These attacks are followed by periods of full or partial recovery, in which no further disease progression is taking place (Vavasour et al., 2022, Lublin and Reingold, 1996, Lublin, 2014).

As stated by Trojano et al. (2003) approximately 90% of the individuals suffering from RRMS convert to SPMS about 20-25 years after its manifestation. SPMS is dominated by neurodegenerative processes such as axonal injury and neuronal loss (Vavasour et al., 2022, Broos et al., 2024). Therefore, it is characterized by a steady progression of MS symptoms after an initial relapsing remitting disease course (Lublin et al., 2014, Lublin and Reingold, 1996). Yet, although attacks and (partial) remission from these attacks can also be observed in SPMS, they do not appear in all individuals (Papiri et al., 2023, Lublin et al., 2014, Lublin and Reingold, 1996).

Another progressive type of MS which is dominated by neurodegenerative processes and activation of the innate immune system of the CNS is PPMS (Vavasour et al., 2022, Papiri et al., 2023, Lublin et al., 2014). Contrary to SPMS, PPMS does not occur after an initial RRMS disease course, but is characterized by disease progression from onset (Lublin et al., 2014, Lublin and Reingold, 1996). Yet, the underlying pathologic mechanism of SPMS and PPMS seems to be similar (Lublin et al., 2014). Overall, PPMS seems to account for 15 to 20 percent of MS cases (Papiri et al., 2023, Hunter, 2016).

An early form, which is considered to be part of the RRMS spectrum and characterized by (multi-)focal inflammation, is CIS (Lublin et al., 2014). According to Thompson et al. (2018), CIS is defined as a monophasic event, in which an individual describes neurologic symptoms for the duration of at least 24 hours that are accompanied by focal or multifocal demyelinating lesions in the CNS but does not fulfil the diagnostic criteria for MS. Furthermore, about 2/3 of the affected individuals progress to RRMS, while about 1/3 remain in an inactive state (Brownlee and Miller, 2014). Due to this, CIS is often considered as the first attack of a RRMS disease course (Thompson et al., 2018, Lublin et al., 2014).

1.1.3 Etiology and Pathophysiology

The etiology and pathophysiology of MS is complex and up until now the subject of research and debate (Attfield et al., 2022). Thus, the following chapter solely aims to give a brief overview of these topics without aiming for a complete coverage. Regarding the etiology, MS is considered to be a multifactorial disorder in which the interaction of an individual's genetic predisposition with environmental factors, cause the disease to emerge (Olsson et al., 2017). In this respect, the most important genetic predisposition lies within certain variations of the human leukocyte antigen class II (HLA II) allele (Moutsianas et al., 2015). For example, according to Moutsianas et al. (2015), heterozygotes for HLA-DRB1*15:01 have an about fourfold increased risk and homozygotes have an about eightfold increased risk of developing MS. Conversely, the same authors showed that certain variations of the HLA class I allele such as HLA-A*02:01 provide protection of MS (Moutsianas et al., 2015). Concerning environmental factors, the Epstein- Barr virus (EBV) is considered to be involved in the emergence of MS either through direct causation of autoimmunity or through the interaction of EBV with predisposing human genes (Aloisi et al., 2023). Other environmental factors supposed to be involved in the etiology of MS are high calory nutrition, obesity, smoking and low vitamin D levels (Nielsen et al., 2017, Jacobs et al., 2021).

Regarding the pathophysiology, MS is characterized by autoimmune inflammatory reactions which cause the disruption of the blood-brain barrier, focal demyelination of axons, death of oligodendrocytes in the white and grey matter as well as axonal and neuronal loss in the CNS (Zierfuss et al., 2024, Lassmann, 2014, Dendrou et al., 2015). The affected areas of

the CNS are referred to as lesions or plaques within which astrocytes form multiple sclerotic glial scars (Dendrou et al., 2015). After the initial inflammatory reaction has subsided, remyelination of previously demyelinated axons can take place (Patani et al., 2007). However, throughout the disease course the degree of remyelination decreases and axonal and neuronal damage accumulate, correlating with degree of sustained disability (Dendrou et al., 2015).

While all phenotypes of MS are characterized by inflammation of the CNS, the acute disruption of the blood-brain barrier with acquisition of new lesions and remyelination is the main feature of RRMS (Lassmann, 2018, Correale et al., 2017). By contrast, progressive forms of MS (PPMS and SPMS) are mostly characterized by the expansion of already existing lesions, resulting in diffuse white matter injury (Elliott et al., 2019). In addition, progressive subtypes show the formation of meningeal inflammatory aggregates, subpial cortical demyelination and brain atrophy (Lassmann, 2018).

The inflammation in multiple sclerosis is induced and maintained by different immune cells such as T-cells, B-cells, macrophages and microglia (Dendrou et al., 2015). Yet, MS is viewed as a mainly T-cell mediated autoimmune disease due to its association with specific HLA class II alleles and the experimental autoimmune encephalopathy in animal models (Weiner, 2004, Sorosina et al., 2023, Fletcher et al., 2010). Further evidence, for MS being a T-cell mediated disease arises from the fact that pharmacotherapy that supresses the migration of T-cells in the CNS such as natalizumab and fingolimod are effective in the treatment of MS (Stuve et al., 2006, Dominguez-Villar et al., 2019). Concerning the inflammatory processes, CD4+ and CD8+ T-cells are migrating across the disrupted blood-brain barrier into the CNS and release proinflammatory cytokines such as interferon gamma and cytotoxic granzyme B (van Nierop et al., 2017, Dias de Sousa et al., 2022, Dendrou et al., 2015). In addition, proinflammatory cytokines activate microglia that damage and destroy oligodendrocytes through oxidative bursts (Lassmann et al., 2012, Dendrou et al., 2015). The arising demyelination causes a dysregulation of ion-channels and oxidative stress within the mitochondria in affected axons, leading to axonal damage (Lassmann et al., 2012). The resulting axonal and cellular debris then lead to a recruitment of further immune cells such as macrophages and B-cells, which in turn cause cytotoxicity through complement reaction and autoantibodies (Lassmann et al., 2012, Franciotta et al., 2008). Concerning the B-cells, especially CD20+ cells are assumed to play a vital role in MS pathology (Lassmann, 2018), which is supported by the effectiveness of CD20+ depleting agents such as ocrelizumab and rituximab (Roos et al., 2023, Franciotta et al., 2008). Results of these pathophysiological immune reactions, such as, oligoclonal bands and the monocyte to lymphocyte ratio in the cerebrospinal fluid serve as important laboratory biomarkers in the diagnosis and prediction of MS progression (Arneth and Kraus, 2022, Huang et al., 2022).

1.1.4 Biomarkers

According to the FDA-NIH Biomarker Working Group (2016), a biomarker is defined as a molecular, histologic or imaging feature that serves as an indicator of healthy or pathogenic biological processes and response to treatment. Thereby, seven types of biomarkers can be distinguished: risk-, diagnostic-, monitoring-, prognostic-, predictive-, treatment response- and safety biomarker (FDA-NIH Biomarker Working Group, 2016, Cagney et al., 2018). Among the many candidate biomarkers of MS that have been identified over the past years, only few could be established that serve as a diagnostic tool in MS subtype identification and a monitor

of disease status as well as clinical response to treatment (Housley et al., 2015). Yet, due to the heterogeneity of the clinical representation of MS, these diagnostic and monitoring biomarkers are highly needed (Arneth and Kraus, 2022). Besides the aforementioned oligoclonal bands, the number and size of white and grey matter lesions assessed through MRI are one of the most important diagnostic, monitoring and response biomarkers of MS (Thompson et al., 2018, Housley et al., 2015). However, although examination of the structural integrity of the CNS has been proven beneficial in the diagnosis of MS (Thompson et al., 2018), it fails to explain what is called the CRP (Barkhof, 2002): the phenomenon that some individuals with MS present themselves with no or few clinical symptoms, although grey and white matter damage is striking and vice versa (Mollison et al., 2017, Dunschede et al., 2023, Barkhof, 2002). Thus, there seems to be a gap between structural integrity and functionality of the CNS. This gap could be closed by the concept of brain reserve, which can be defined as the degree of cortical plasticity that allows the CNS to compensate for neural damages (Tremblay et al., 2023, Machado et al., 2021, Esiri and Chance, 2012). Accordingly, the depletion of the brain reserve by means of a reduced cortical plasticity may cause loss of CNS functionality and MS progression (Mori et al., 2013). Consequently, introducing the degree of cortical plasticity as a surrogate biomarker for MS may, among other things, help diagnosing the individual's subtype of MS, evaluate their risk of disease progression and response to treatment. Regarding cortical plasticity, LTP-like plasticity, seems to play a crucial role in MS, due to its ability to compensate for neuronal and axonal loss through increased excitation and reconnection of the remaining neurons (Stampanoni Bassi et al., 2017, Stampanoni Bassi et al., 2019b).

1.1. The Role of LTP-Like Cortical Plasticity

LTP-like cortical plasticity has many facets, but in essence can be defined as the increment of synaptic signaling and strengthening after synchronized high frequency stimulation of glutaminergic neurons in the CNS (Nicoll and Roche, 2013). Although there are many mechanisms through which LTP-like plasticity is facilitated, the most prominent way is the glutaminergic excitement of N-Methyl-D-Aspartate (NMDA) receptors, which results in an influx of calcium ions (Lu et al., 2001). This influx causes the activation of kinases, which lead to the recruitment of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to the synapse (Luscher and Malenka, 2012, Lu et al., 2001). The counterpart of LTP is LTD, which is defined as the decrement of synaptic glutaminergic signaling after low frequency neuronal stimulation and is associated with endocytosis of AMPA receptors (Hanley, 2018).

The degree of LTP-like cortical plasticity seems be affected by MS. For example, Mori et al. (2014a) demonstrated that LTP-like plasticity is reduced in MS patients with partial or absent recovery after an MS attack, but not in those with full recovery. In this context, Stampanoni Bassi et al. (2019b) revealed that the reduction in LTP-like plasticity in individuals with RRMS correlates with high levels of pro-inflammatory cytokines such as interleukin-6. Moreover, according to the authors, interleukin-6 is elevated in the CSF of RRMS patients with newly acquired lesions and those with longer disease duration before treatment commencement (Stampanoni Bassi et al., 2019b). This suggests that LTP-like plasticity might be a predictor of lesion load and disease activity. Further support for this claim comes from studies, showing that elevated interleukin-1β levels in the CSF of individuals with RRMS and elevated tumor necrosis factor-α (TNF-α) levels in the CSF of individuals with PMS are associated with

glutaminergic excitotoxicity and neurodegeneration and reduced LTP-plasticity (Rossi et al., 2014, Rossi et al., 2012, Rizzo et al., 2018) In addition, a meta-analysis conducted by Karimi et al. (2022) revealed that brain-derived neurotropic factor (BDNF), which is irremissible in the maintenance of LTP (Vignoli et al., 2016), is reduced in the plasma of MS patients.

Consequently, it seems that the degree of LTP-like cortical plasticity reflects the amount of neuroinflammation and neurodegeneration in individuals with MS. Therefore, it could be proposed that LTP-like cortical plasticity represents an eligible biomarker, for the diagnosis of MS subtype, prediction of MS progression and response to treatment. A way to non-invasively induce LTP in humans is rTMS (Taylor et al., 2018, Agboada et al., 2020).

1.2 Transcranial Magnetic Stimulation

1.2.1 Basic Principles

Since the 1980s, the conductance of non-invasive brain stimulation, especially of the primary motor cortex (M1), using transcranial magnetic stimulation (TMS) gained increasing popularity in brain research (Miron et al., 2021). TMS facilitates brain stimulation through the discharge and conductance of a brief electromagnetic current through the skull, which causes a depolarization of the neurons in the target area (Barker et al., 1985). The propagation of this evoked excitation results in neurophysiological and behavioral changes, making TMS a powerful tool to assess CNS functionality (Martel and Glover, 2023, Agboada et al., 2020). In that regard, single pulse TMS, a stimulation pattern in which an electromagnetic pulse is released at an interval between four to eight seconds (s), is frequently used (Klomjai et al., 2015, Jannati et al., 2023). For example, applied over the M1, single pulse TMS causes a depolarization of pyramidal neurons either directly or indirectly through the depolarization of local interneurons (Terao et al., 2000, Rusu et al., 2014, Hamada et al., 2013). This depolarization is then propagated along cortico-cortical and corticospinal pathways and results in a contraction of the targeted contralateral muscle (Klomjai et al., 2015, Ferreri et al., 2011, Barker et al., 1985). Using electromyography (EMG), this muscle contraction can be assessed by means of motor evoked potentials (MEPs) (Klomjai et al., 2015, Barker et al., 1985). In this respect, studies revealed that TMS evoked MEPs are a suitable measure to assess corticospinal and cortico-cortical tract integrity in humans (Manogaran et al., 2016, Jannati et al., 2023).

Unlike single pulse TMS, which is used to investigate the functionality of the brain as described above, rTMS is used to induce transient changes in neuronal excitability through changes in cortical plasticity (Klomjai et al., 2015). rTMS induces these changes through the generation of a series of brief electromagnetic pulses, which depending on the frequency measured in hertz (Hz), result in either LTP ($\geq$ 5 Hz) or LTD ($\leq$ 1 Hz) (Fitzgerald et al., 2006). With regard to the stimulation of the M1, LTP is reflected by an increment and LTD by a decrement in the MEP amplitude (Delvendahl et al., 2012). These changes through rTMS are generally referred to as after effects (Klomjai et al., 2015). The duration of these after effects depend on many factors such as the stimulation intensity (Lang et al., 2006), stimulation frequency (Klomjai et al., 2015), the number of stimuli (Gilio et al., 2007) and the degree of contraction of the target muscle during stimulation (Fujiwara and Rothwell, 2004). The stimulus intensity is traditionally based on the resting motor threshold (RMT) (Turi et al., 2021), or on the active motor threshold (AMT) (Turi et al., 2021). To this respect, RMT is defined as the lowest stimulation intensity resulting in an MEP bigger than, or equal to 50 microvolts (µV) in 5 out of 10 trials of single pulse stimulation (Rothwell et al., 1999). Similarly, AMT is

defined as the lowest stimulation intensity resulting in an MEP of ≥ 100µV in 5 out of 10 single pulse stimulation trials during slight target muscle contraction (Muellbacher et al., 2000, Di Lazzaro et al., 2000). In general, LTP and therefore MEP increments are facilitated when the stimulation intensity (van den Bos et al., 2017), the stimulation frequency (Fitzgerald et al., 2006) as well as the number of stimuli is high (Gilio et al., 2007) and when the stimulus is preceded by contractions of the target muscle (van den Bos et al., 2017). Conversely, LTD and, therefore, MEP decrements are only occurring when the target muscle is at rest (Goldsworthy et al., 2015) and facilitated when the ≤ 1 Hz stimulus is preceded by a high frequency sub-threshold stimulus (Iyer et al., 2003).

The summation of these stimulation characteristics is specified in a rTMS protocol (Klomjai et al., 2015). Over the past years, different LTP inducing rTMS protocols have emerged and been applied to plasticity research (Wittkopf et al., 2021, Uygur-Kucukseymen et al., 2023). In the search for new MS biomarkers, these protocols show high potential, because they are not only non-invasive (Barker et al., 1985), timesaving and inexpensive (Voigt et al., 2017), but also facilitate the real time mapping of the CNS functionality through the assessment of cortical excitability in relation to clinical symptoms by means of e.g., MEP amplitude and MEP latency (Mori et al., 2013). The latter is defined as the time interval between the application of the TMS stimulus and MEP onset and therefore, represents the corticomotor conductance time of the pyramidal tract (Groppa et al., 2012, Cacchio et al., 2011).

1.2.2 rTMS Protocols and LTP-like Plasticity Induction in MS

In this paragraph, three of the most frequently used rTMS protocols of the M1 and their role in LTP-like plasticity induction in MS will be presented. A commonly used rTMS protocol in the stimulation of the M1 is paired associative stimulation (PAS) (Suppa et al., 2017, Stefan et al., 2000). This protocol is characterized by the pairing of repetitive electrical stimulation of somatosensory afferents such as the median nerve with rTMS of the contralateral M1 (Stefan et al., 2000). Due to the stimulation of two targets, the after effects of PAS are based on spike-timing-dependent plasticity (Müller-Dahlhaus et al., 2010, Hernandez-Pavon et al., 2023a). This means that due to the delay of the afferent stimulus transmission compared to direct stimulation of the first M1, LTP-like or LTD-like after effects depend on the timing between the two stimuli: the interstimulus interval (ISI) (Stefan et al., 2000, Hernandez-Pavon et al., 2023a). If the stimulation results in a synchronous activation of neurons in the M1, that is ISI of about 25 milliseconds (ms), PAS induces LTP (Suzuki et al., 2023). However, if the stimulation is asynchronous, that is ISI of 10 ms or less, PAS induces LTD (Weise et al., 2013).

Regarding MS plasticity research, Zeller et al. (2010) demonstrated that there was no difference in LTP-like plasticity between individuals with stable MS and healthy controls (HC) after the application of PAS to the M1 with an ISI of 25 ms. However, this null-finding may be due to the overall mildly affected sample of individuals with stable MS in which neurodegenerative und inflammatory processes that impede LTP-like plasticity are not as pronounced (Stampanoni Bassi et al., 2019b, Rossi et al., 2014). Indeed, using a similar protocol, Mori et al. (2014a) demonstrated that the application of PAS to the M1 resulted in higher degrees of LTP-like plasticity in individuals with stable RRMS compared to those, who were in relapse or partial remission. Yet, Conte et al. (2009) did not find differences in PAS-induced LTP-like plasticity between individuals with SPMS and individuals with RRMS or HC and Conte et al. (2016) found a reduced PAS-induced LTP-like plasticity in individuals with

RRMS. While these findings may have been influenced by small sample size and lacking power, a general problem with PAS and other rTMS protocols is the high number of non-responders as well as a high degree of variability and thus, low intra- and interindividual reliability (Minkova et al., 2019, Guerra et al., 2020a, Fratello et al., 2006).

Another frequently used rTMS protocol in MS research is theta burst stimulation (TBS) (Huang et al., 2005). TBS is a high frequency protocol of 50 Hz, in which a train of three TMS bursts is applied at an ISI of 5 Hz (Huang et al., 2005), mimicking the natural hippocampal theta rhythm of 4 to 7 Hz (Ford et al., 1970, Diamond et al., 1988). According to Huang et al. (2005), two forms of TBS can be distinguished: LTD inducing continuous TBS (cTBS) and LTP inducing intermittent TBS (iTBS). While cTBS follows the pattern described above, iTBS is interspersed with ten second breaks after administration periods of two seconds (Huang et al., 2005). An advantage compared to other rTMS protocols is that TBS can produce large LTP-like effects after two to three minutes of administration (Huang et al., 2005), compared to 30 minutes in e.g. PAS protocols (Stefan et al., 2000), making it more "patient friendly".

Concerning MS plasticity research, Mori et al. (2013) demonstrated that iTBS induces equal degrees of LTP-like plasticity in HC and mildly affected individuals with stable RRMS. These findings are concordant with those of Zeller et al. (2010) and may provide further evidence that neuroinflammation und neurodegenerative processes that disturb LTP-like plasticity are not prominent in the early and stable phases of the RRMS disease course (Stampanoni Bassi et al., 2019b, Rossi et al., 2014). In line with that assumption, Mori et al. (2012) demonstrated that individuals with RRMS who were in relapse showed lower degrees of LTP-like plasticity in response to iTBS, than stable RRMS individuals and that this difference wore off after six months. Moreover, Mori et al. (2013) demonstrated that compared to individuals with stable RRMS and HC, individuals with PPMS had significantly lower degrees of LTP-like plasticity. Taken together, these findings suggest that TBS might in fact detect clinical progression in MS, which should further be investigated through longitudinal research.

However, Mori et al. (2013) also demonstrated paradox TBS results in which cTBS resulted in LTD-like plasticity in HC and LTP-like plasticity in individuals with stable RRMS. This might lead to the conclusion that plasticity in MS is not reduced, but rather dysregulated. Yet, it has to be taken into account that TBS is, like PAS, characterized by a high non-responder rate, as well as low interindividual and moderate intraindividual reliability, which can result in fluctuating and conflicting results (Corp et al., 2020). Nevertheless, for rTMS to be a suitable biomarker for MS, measurements need to be reliable and valid (Mayeux, 2004). However, new protocols aiming to solve these problems are already on the rise.

Such a promising TMS protocol is quadripulse stimulation (QPS). QPS was first introduced by Hamada et al. (2007) and consists of the repetitive application of trains of four monophasic TMS stimuli. Like TBS and PAS, QPS is assumed to induce LTP-like plasticity in the M1 mainly through the activation of glutamatergic excitatory synapses (Matsumoto and Ugawa, 2020). In that regard, Hamada et al. (2008) showed that the application of 360 QPS bursts at the left M1 with a stimulus intensity of 90 percent AMT over the period of 30 minutes at an ISI of five ms (200 Hz) and an interburst interval (IBI) of five seconds (0.2 Hz) induced a powerful increase of MEPs of the left first dorsal interosseus muscle for 75 minutes (Hamada et al., 2008). Moreover, it could be demonstrated, that QPS induces higher degrees of LTP-induction in HC than iTBS, with less variability and lower non-responder rates (Tiksnadi et al., 2020, Nakamura et al., 2016). Therefore, QPS might be a suitable instrument to reliably assess

LTP-like cortical plasticity in MS patients and serve as a biomarker for the diagnosis of MS subtype, disease progression and response to treatment.

Indeed, within our research group, Balloff et al. (2022), recently demonstrated in an RRMS sample that QPS-induced increments in MEP amplitude significantly negatively correlated with scores on the expanded disability status scale (EDSS) and significantly positively correlated with the degree of visuospatial short-term memory and learning as well as information processing speed. In this context, it was found that while the overall mildly affected RRMS sample did show normal degrees of LTP-like plasticity, cognitively impaired RRMS individuals did not (Balloff et al., 2022). To that respect, it was demonstrated that individuals with RRMS who showed higher degrees of cognitive impairment had lower increments in MEP amplitude and thus, showed lower degrees of LTP-like plasticity than those, who were less cognitively impaired (Balloff et al., 2022). These findings suggests that QPS-induced LTP-like plasticity can detect clinical aggravation in MS. However, a subsequent study by our research group, showed no significant difference in QPS-induced LTP-like plasticity between HC, individuals with stable RRMS and individuals with RRMS, who were in relapse (Balloff et al., 2023b). Yet, in that study, non-significant trends showed, that compared to the other groups, relapsing individuals had a lower and slower increase in MEP amplitude and thus, impaired LTP-like plasticity (Balloff et al., 2023b). In addition, due to the fact that assessment of Balloff et al. (2023b) took place with an average delay of 21 days after relapse onset and after therapeutic intervention, the inflammatory reaction that impedes LTP-like plasticity may have already been attenuated in some individuals (Steinman, 2014). This, in conjunction with the medium sample size of 18 individuals per group may have resulted in insufficient power to demonstrate an effect. Another finding by our research group that seems somewhat paradox is that a subgroup of relapsing RRMS individuals who presented disability had significantly higher MEP amplitudes and thus, higher degrees of LTP-like plasticity than a subgroup of relapsing RRMS individuals without disability (Balloff et al., 2023b). The hypothesis that this finding may have been caused by excitotoxicity and therefore increased glutamate release was discarded by the authors, because MEP amplitudes of disabled individuals did not exceed those of healthy individuals (Balloff et al., 2023b). However, given the observed trends, another way of interpretation could be that excitotoxicity in an overall LTP-like plasticity impaired group of disabled relapsing individuals increased MEP-amplitudes to a normal level (Sarchielli et al., 2003, Rossi et al., 2012, Abdel Naseer et al., 2020).

Taken together, QPS-induced LTP-like plasticity seems to be a promising candidate biomarker of MS. Still, previous findings are limited to RRMS samples and need to be replicated due to partly insufficient power. In addition, findings provided by other rTMS protocols on the degree of LTP-like cortical plasticity in individuals with PMS have so far been inconsistent and are based on underpowered study designs (Mori et al., 2013, Conte et al., 2009). However, to introduce QPS-induced LTP-like plasticity as a diagnostic, prognostic and monitoring biomarker for MS, its ability to distinct between other subtypes of MS, including PMS, needs to be investigated (Cagney et al., 2018).

1.3 Aims and Hypotheses

This thesis project was based on an ongoing longitudinal research project focussing on changes in cortical plasticity, as well as cognitive functioning and motor functioning in MS patients over time. Due to its longitudinal design, this research project was, and still is, the

subject of other doctoral theses. All of these theses had or still have their own research questions, aims and hypotheses.

The aim of this thesis was to investigate whether QPS-induced LTP-like plasticity can discriminate between individuals with PMS and RRMS, as well as healthy individuals, in order to establish its suitability as a biomarker of MS. For that purpose, a study containing a QPS protocol which was based on Hamada et al. (2008), but in accordance with Nakamura et al. (2016), used an extended assessment time of LTP-like cortical plasticity, was conducted across three groups of participants: PMS, stable RRMS and healthy individuals. LTP-like plasticity was measured at one pre-QPS and six post-QPS assessments by means of the amplitude of 12 rTMS triggered MEPs of the right first dorsal interosseus muscle (FDI). To determine differences in the increment of LTP-like plasticity between the three groups, the highest mean post-QPS MEP amplitude was compared with the mean pre-QPS MEP amplitude. Moreover, to assess the degree of disability and other traits associated with reduced cortical plasticity, this protocol was accompanied by a neurological examination and a neuropsychological test battery as well as the assessment of the corticomotor conductance time by means of the MEP latency.

In line with the previous findings of our research group, published by Balloff et al. (2022) and Balloff et al. (2023b), differences in QPS-induced LTP-like plasticity were not expected to differ between healthy individuals and mildly affected stable RRMS individuals. The reason behind this assumption is that inflammatory as well as neurodegenerative processes interfering with LTP-like plasticity in the latter group are less extensive (Stampanoni Bassi et al., 2019b, Luchetti et al., 2018). Conversely, neurodegenerative processes as well as chronic inflammation have been shown to be prominent in individuals with PMS (Luchetti et al., 2018, Kallaur et al., 2017). Hence, it was assumed that individuals with PMS show lower degrees of QPS-induced LTP-like plasticity compared to individuals with both, mildly affected stable RRMS and healthy individuals. Based on these assumptions, the following hypotheses (H) were stated:

1. QPS induces cortical plasticity in all groups (H_1).
2. Individuals with stable RRMS do not differ in their degree of QPS-induced LTP-like plasticity from healthy individuals (H_2).
3. Individuals with PMS have lower degrees of QPS-induced LTP-like plasticity than individuals with stable RRMS (H_{3a}) and healthy individuals (H_{3b}).

2. Methods

2.1 Ethical Approval and Data Protection

The ethics committee of the medical faculty of the University of Düsseldorf approved the study on the 5th of May 2018 under the reference number 2018-16. In accordance with the declaration of Helsinki, all subjects gave their written informed consent prior to their participation in this study. Moreover, all participants signed a data use agreement, conforming with the European General Data Protection Regulation.

2.2 Participants

2.2.1 Participant Characteristics

In this interventional, cross-sectional, prospective study n = 34 individuals with PMS (PPMS: n = 14, SPMS: n = 20) were matched in age, sex and education with n = 30 HC and n = 30 individuals with RRMS. Diagnosis of MS subtype and the allocation to a subgroup of MS

(RRMS, PPMS and SPMS) was based on the 2017 revised McDonald criteria (Thompson et al., 2018). Across this entire sample of $N = 94$ participants, age ranged between 30 and 70 years with $M = 49.72$ years ($SD = 8.33$) and $Mdn = 51$ years ($IQR = 11.75$). Moreover, $n = 48$ (51.06 %) of the sample were female and $n = 46$ (48.94 %) were male, with an overall length of education of $M = 15.98$ years ($SD = 2.94$) and $Mdn = 16$ years ($IQR = 5.5$).

If needed, all participants received a free parking ticket for on campus parking and no other financial reward was given for participation. However due to the lack of HC above the age of 40, a reward of 30 Euros was offered from March 2021 onwards for participants meeting these characteristics ($n = 7$). The inclusion and exclusion criteria, the procedure of the recruitment of eligible participants, as well as the matching of participants is discussed in detail in the corresponding sections below.

2.2.2 Inclusion and Exclusion Criteria

The selection of eligible participants was based on predefined inclusion/exclusion criteria and is depicted in **Figure 1**. The inclusion criteria were (1) being between the age of 18 and 80 years, (2) sufficient knowledge of the German or English language, (3) having sufficient or sufficiently corrected visual acuity to read instructions and absolve neuropsychological testing and (4) confirmed diagnosis of multiple sclerosis of the RRMS, PPMS or SPMS subtype, according to the 2017 revised McDonald Criteria (Thompson et al., 2018). Exclusion criteria were assessed using a standardized questionnaire, which also contained items from the TMS safety screener developed by Rossi et al. (2011). Exclusion criteria included, A) the lack of the individual's general ability to give informed consent: (1) age under 18 and (2) having a legal guardian. B) any contraindications for rTMS as stated by Di Iorio and Rossini (2017) and Wassermann (1998): (3) being pregnant, (4) having epilepsy or a history of seizures, (5) having had unexplained syncope, (6) having or having had a concussion, (7) having or having had meningitis, (8) having or having had a brain tumour or stroke, (9) having any kind of metal implants or fragments in brain or skull, (10) having any deep brain stimulating device, cardiac pacemaker or cardioverter defibrillator, (11) having any kind of non-detachable infusion pump, (12) having a cochlear implant or (13) having experienced any adverse events linked to previous TMS treatment or MRI. C) any medical condition or intake of any medication/substance resulting in altered brain excitability, which might interfere with the experimental manipulation: (14) having a neurological illness other than RRMS, PPMS or SPMS (Bologna et al., 2017, Kuhn et al., 2004), (15) having experienced an MS relapse less than 30 days prior to assessments (Wirsching et al., 2018), (16) having any psychiatric disorder requiring treatment at the time of assessment (Radhu et al., 2013), with the exception of remitted depressive episodes, due to high prevalence of this condition in individuals with MS (Boeschoten et al., 2017), (17) any kind of substance abuse or substance use disorder (Kaarre et al., 2018, Martin-Rodriguez et al., 2021), (18) regular intake of dextromethorphan or nimodipine (Wankerl et al., 2010) and (19) intake of triptans less than two weeks prior to assessment (Becerra et al., 2016). However, consistent with the previous research of our research group (Balloff et al., 2022), even if the abovementioned criteria for participation were satisfied, individuals were excluded, when they did not show sufficient cortical excitability, defined as a mean MEP amplitude of ~ 0.5 mV after ten consecutive trials of TMS prior to baseline assessment (Tsutsumi et al., 2014).

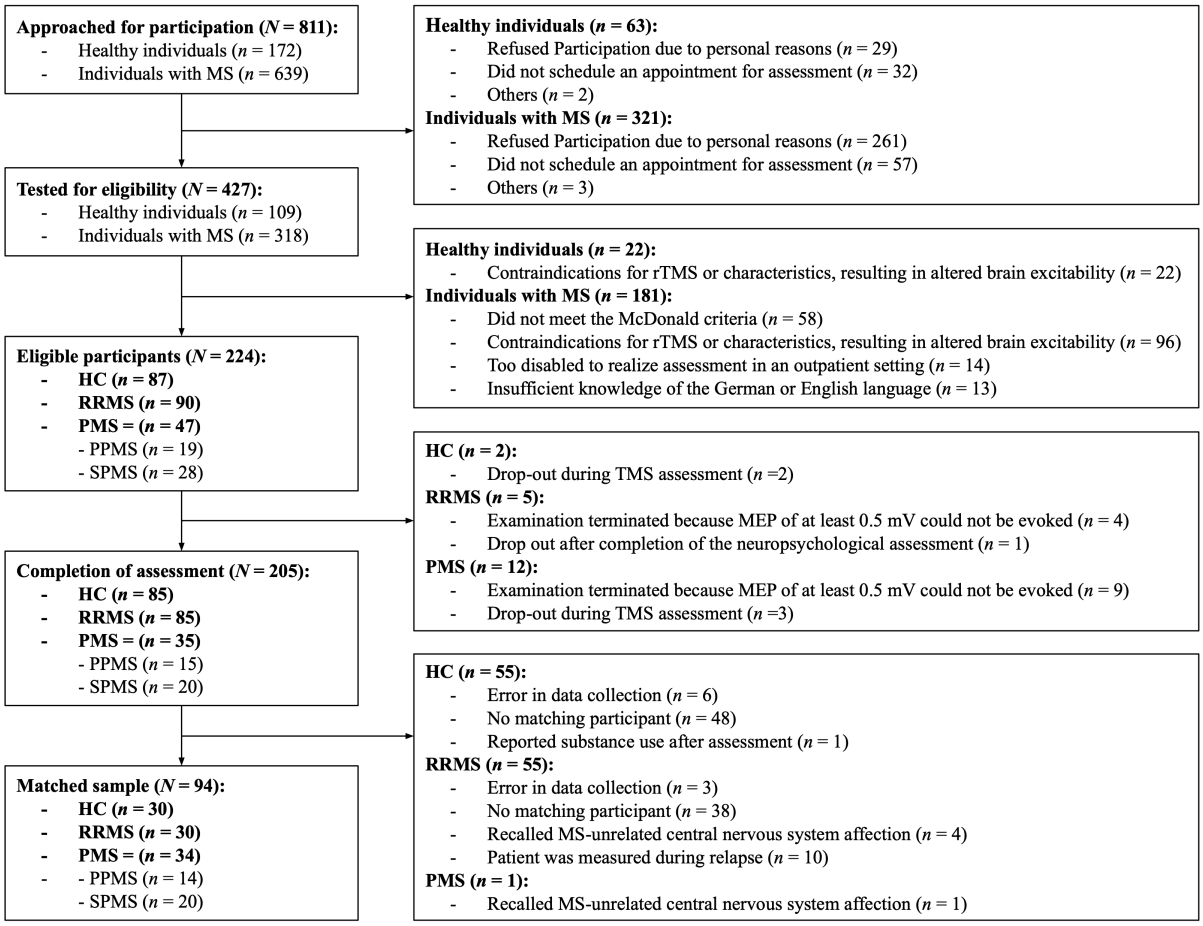

Fig. 1: Selection Process and Matching of Participants.

This flowchart depicts the selection process and matching of participants. The selection was based on predefined inclusion and exclusion criteria. Firstly, inpatients, outpatients and healthy individuals were approached for their willingness to participate. When they expressed their interest, they were tested for eligibility. In addition, the MS diagnosis and subgroup membership of individuals with MS was confirmed by a clinical examination. Matching of participants in age, sex, as well as level and years spent in education, resulted in the final sample. Abbreviations: HC, healthy controls; MEP, motor evoked potential; MS, multiple sclerosis; PMS, progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis; RRMS, relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; TMS, transcranial magnetic stimulation. *Note*. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 04. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

2.2.3 Matching of Participants

Participants in the PMS group were matched in age and sex with individuals in the RRMS group and HC (**Figure 1**). However, if more than one individual in the pool of potential HC or individuals with RRMS satisfied these two criteria, concordance in the level of education, as assessed defined by the German version of the Consortium to Establish a Registry for Alzheimer's Disease neuropsychological test battery (CERAD) (Memory Clinic, n.d., Thalmann et al., 1998) described in detail below, determined matching. To reduce bias, the researcher who conducted matching of participants was not aware of the rTMS results or those clinical traits not relevant for matching. As this cross-sectional study is part of a longitudinal study (reference number 2018-16), baseline (n = 32) as well as follow-up (n = 2) measurements of participants in the PMS group collected from May 2018 until October 2022 were matched

with baseline (n = 24) or with follow-up (n = 6) measurements of participants in the RRMS and with baseline (n = 23) or with follow-up (n = 7) measurements of participants in the HC group.

2.2.4 Recruitment Procedure

The recruitment of eligible participants took place from May 2018 until October 2022. However, recruitment and assessment were paused from September 2018 till March 2019 due to lack of staff and from September 2021 until April 2022 due to breaking down of the rTMS coils and maintenance work of the TMS stimulator. Individuals with MS were recruited either during outpatient appointments at the interdisciplinary outpatient chemotherapy centre and the neurological outpatient clinic, or during their inpatient treatment at the neurology ward at the University Hospital Düsseldorf (UHD), Germany. Recruitment was conducted by physicians, doctoral students and student research assistants working at the department of neurology at the UHD. The aim was to ask all MS patients present at any given day in the above-mentioned facilities for their willingness to participate. However, due to workload and work hours of the staff, it was not possible to realise this on each day of the recruitment period. Firstly, all patients were informed verbally about the study and its procedure. If patients were interested in participating, they also received printed information material about the study, including a brief description of the research question and study design, the procedure, inclusion and exclusion criteria, informed consent and contact information. Outpatients were then given seven days to read the information material before they were contacted again via phone, to assess whether they were still interested in participating, to systematically check inclusion and exclusion criteria and if all criteria were met, schedule an appointment for assessment. Unlike outpatients, inpatients were contacted again 24 hours after printed information was given, with the aim of realising assessment during their regular stay at the hospital.

HC were recruited among university students, hospital staff as well as family members and friends of participants with MS and researchers using flyers. However, a lack of HC over the age of 40 led to problems in matching participants. Thus, additional healthy individuals meeting these characteristics were randomly recruited by phone from a database from previous rTMS studies at the department of neurology at UHD, form March 2021 onwards.

2.3 Materials

2.3.1 Electromyographic Recording

The TMS set-up is depicted in **Figure 2**. MEPs were recorded using surface electromyography (EMG). Therefore, two Ag/AgCl surface electrodes of 20x15 mm (Ambu® Neuroline™ 700, Ballerup, Denmark) were positioned in a belly tendon montage on the right FDI and one Ag/AgCl ground electrode of 48x30 mm (Ambu® Neuroline™ Ground (714), Ballerup, Denmark), was placed on the right inner forearm. To record the signal, the three electrodes were connected with a signal-conditioning amplifier (Digitimer D360, Digitimer Ltd, Hertfordshire, UK) by three 150 centimeters (cm) long lead wires with DIN42802 connectors and 0.7 mm sockets on the opposite side (Spes Medica, Genova, Italy). The recorded signal was led through and filtered by this signal-conditioning amplifier with a bandwidth of 100-5000 Hz, collected at a sampling rate of 5 kilohertz (kHz) and real time monitored, using an oscilloscope (DS1074B, Batronix Rigol, Preetz, Germany). The collected EMG data was stored on a computer and analysed using Signal version 6.02 (Cambridge Electronic Design Ltd., Cambridge, UK).

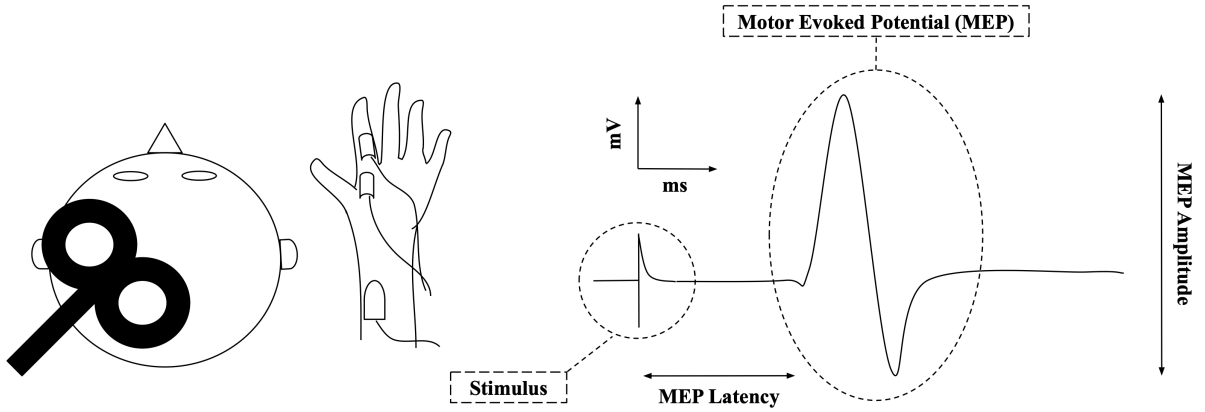

Fig. 2: Experimental rTMS Set-Up.
This illustration depicts the experimental rTMS set-up. rTMS was performed to the hand area of left M1 in order to evoke MEPs and assess MEP latency of the right FDI. Both were facilitated using a figure-of-eight shaped, hand-held magnetic coil, which was placed tangentially to the skull at a 45-degree angle to the sagittal plane with the handle pointing posteriorly. Electromyography was used to record MEPs and MEP latencies. Therefore, two surface electrodes were positioned in a belly tendon montage on the right FDI and one ground electrode was placed on the right inner forearm. MEP amplitude was measured from peak to peak in mV and MEP latency, the time between stimulus induction and MEP elicitation, was measured in ms. Abbreviations: FDI, first dorsal interosseus muscle; M1, primary motor cortex; MEP, motor evoked potential; mV, millivolts; ms, milliseconds. *Note*. Adapted from "Long-term potentiation-like plasticity is retained during relapse in patients with Multiple Sclerosis" by C. Balloff, S. Novello, A.-S. Stucke, L.K Janssen, E. Heinen, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner, P. Albrecht, S.J. Groiss, 2023, *Clinical Neurophysiology*, 155, p. 79. (https://doi.org/10.1016/j.clinph.2023.07.013). Copyright 2023 by Elsevier B.V..

2.3.2 Repetitive Transcranial Magnetic Stimulation

In this study, a stimulus protocol was used which was based on Hamada et al. (2008), but in accordance with Nakamura et al. (2016), used an extended assessment time of LTP-like cortical plasticity that spanned a duration of one hour. Cortical plasticity was measured by means of the magnitude of the MEP amplitude of the right first dorsal interosseous (FDI) elicited through rTMS before (pre-QPS assessment) and after QPS administration (post-QPS assessment). In that connection, and in line with the previous work of our group (Balloff et al., 2022), LTP-like cortical plasticity was defined as the increment in the magnitude of the MEP amplitude from pre-QPS to post-QPS assessment (Hamada et al., 2008). While this section aims to describe how rTMS was facilitated, the rTMS stimulus protocol and QPS will be described in detail in the corresponding section below.

To evoke MEPs of the right FDI, single pulse monophasic rTMS with a stimulus frequency of 0.2 Hz was performed to the hand area of left M1, using a figure-of-eight shaped, hand-held magnetic coil with an outer diameter of 70 millimetres (mm) (The Magstim Company Ltd., Whitland, UK). The magnetic coil was connected to a Magstim BiStim^2 stimulator (The Magstim Company Ltd., Whitland, UK) and was placed tangentially to the skull at a 45-degree angle to the sagittal plane with the handle pointing posteriorly (**Figure 2)**. The position of the hand area of the left M1 was estimated with a measuring tape and marked with a pen by measuring 5 cm to the left and 1 cm ventrally from the vertex of the skull (Topka, 2007), which is defined as the point of interception between the line connecting inion and nasion and the line connecting the tragus of both ears. From that estimated position, the target site, also referred to as the "motor hot-spot", defined as the point resulting in the highest motor evoked potentials (MEPs) of the relaxed right FDI after TMS stimulation (Kaelin-Lang, 2007),

was located using single pulse monophasic TMS at an individual stimulus intensity (Groppa et al., 2012). For that purpose, the TMS coil was displaced in the anterior-posterior or medial-lateral direction in steps of 1 cm or less, until the motor hotspot was detected (Groppa et al., 2012, Claus, 2007). The location of the motor hot-spot was marked with a pen, to guarantee the constant position of the TMS coil throughout the assessment.

2.3.3 rTMS Stimulus Protocol

As mentioned above, this cross-sectional study was based on an ongoing longitudinal research project, which focusses on changes in cortical plasticity, cognitive and motor functioning in MS patients over time. To assess the various degrees of neural plasticity, a stimulus protocol comprising six different stimulus intensities was specified: 100% RMT, 110% RMT, 120% RMT, 130% RMT, 140% RMT and MEP 0.5 mV.

Stimulus intensities that were based on the individuals RMT were calculated as a percentage, using a calculator. RMT was determined at the motor hot-spot of each individual. In accordance with Rothwell et al. (1999), it was defined as the lowest stimulus intensity, eliciting a MEP peak-to-peak amplitude of $\geq$ 50µV in five out of ten consecutive TMS trials in the resting right FDI, when applying a relative frequency method. These trials were administered at a frequency of 0.2 Hz. After a general estimation, RMT was approximated by altering the stimulus intensity in 1% steps of the maximum stimulator output.

Like RMT, the MEP 0.5 mV threshold for each individual was defined as the stimulus intensity eliciting a peak-to-peak amplitude of about 0.5 mV at the motor hotspot in 10 consecutive trials, when applying a relative frequency method (Groppa et al., 2012, Balloff et al., 2022)**.** Yet, due to fluctuation in MEP amplitude, some deviation from this value was tolerated, while maintaining an average MEP amplitude of about 0.5 mV for each group. Although, all six stimulation intensities were included in the stimulation protocol of this study, only the stimulus intensity eliciting a MEP amplitude of about 0.5 mV at pre-QPS assessment was of interest (Hamada et al., 2008, Balloff et al., 2022).

In this study, rTMS stimuli were applied in seven blocks which were separated by 4-minute breaks: pre-QPS, post-QPS_1, post-QPS_2, post-QPS_3, post-QPS_4, post-QPS_5 and post-QPS_6. Each block consisted of a total of 72 stimuli, which in turn consisted of 12 stimuli for each of the 6 different stimulus intensities (100% RMT, 110% RMT, 120% RMT, 130% RMT, 140% RMT and MEP 0.5 mV). Within each block, stimuli of different intensities were applied in a random manner using Signal version 6.02 (Cambridge Electronic Design Ltd., Cambridge, UK) at a frequency of 0.2 Hz. Thus, each block had a duration of six minutes.

Stimulus responses were discarded when amplitudes were contaminated by voluntary movement or other measurement artefacts (Hamada et al., 2008, Balloff et al., 2022). The remainder of the 12 amplitudes of the stimulus intensity MEP 0,5 mV were averaged for each block (pre-QPS, post-QPS_1, post-QPS_2, post-QPS_3, post-QPS_4, post-QPS_5 and post-QPS_6). For statistical analysis, the average MEP amplitude during pre-QPS assessment was only compared to the post-QPS assessment block with highest average MEP Amplitude (post-QPS_{max}). This is because the latter was assumed to reflect the maximum gain in cortical excitability due to an increase in LTP-like plasticity after QPS administration (Balloff et al., 2022).

2.3.4 Quadripulse Stimulation

LTP-like cortical plasticity was induced using a QPS protocol, which was based on Hamada et al. (2008). In accordance with this protocol, QPS was facilitated by connecting four magnetic stimulators (Magstim 2002, The Magstim Company Ltd., Whitland, UK) with a combining module (The Magstim Company Ltd., Whitland, UK), allowing for the application of a train of four monophasic TMS pulses through one TMS coil. Thereby, the coil itself as well as its position on the skull during QPS was equal to single pulse monophasic rTMS described above. Following the protocol of Hamada et al. (2008), stimulation intensity was set to 90% AMT. AMT was defined as the stimulus intensity required to elicit a MEP peak-to-peak amplitude of $\geq$ 100µV during 10-20% of maximum contraction of the right FDI in five out of ten consecutive TMS trials, when applying a relative frequency method (Muellbacher et al., 2000, Di Lazzaro et al., 2000). AMT approximation was conducted in the same way as RMT approximation. Furthermore, in line with Hamada et al. (2008), QPS was administered using 360 TMS bursts with an ISI of 5 ms and an inter-train frequency of 0.2 Hz (Hamada et al., 2008). This resulted in a total administration of 1440 magnetic stimuli over a period of 30 minutes. During QPS administration the right FDI was relaxed and all MEPs were monitored.

2.3.5 MEP Latency

MEP latency, which represents the time of corticomotor conductance (Groppa et al., 2012), was measured to assess the integrity of the corticospinal pathway (McKay et al., 1997, Firmin et al., 2012). MEP latency was defined as the time interval between stimulus application through TMS and MEP onset: MEP onset – onset of TMS stimulus application in ms (Cacchio et al., 2011, Shulga et al., 2015). In that connection, MEP onset was defined as the first TMS stimulus associated deviation from the horizontal EMG trace prior to the first MEP peak (Cacchio et al., 2011). In accordance with previous research by our group, MEP latency was assessed using 10 monophasic TMS stimuli with a frequency of 0,2 Hz and a stimulus intensity of 140% AMT, while right FDI contraction of about 30% of the maximum force was maintained (Balloff et al., 2022). The stimulus intensity was calculated as a percentage, using a calculator. Out of the 10 MEP latencies, the mean MEP latency was calculated for each individual. In accordance with previous research by our group, MEP latencies below 24.5 milliseconds were considered normal and those above 24.5 milliseconds were considered pathological Balloff et al. (2023a).

2.3.6 Neuropsychological Test Battery

Due to it being part of a longitudinal research project, this cross-sectional study included an extensive neuropsychological test battery. In this study, however, only the assessment of depressive symptoms, anxiety symptoms, fatigue, information processing speed as well as visuospatial short-term memory and learning were of interest, because they are either directly associated with cortical plasticity or can be considered as potential confounders (Balloff et al., 2022). The corresponding tests will therefore be described in detail below, while the others will only shortly be listed to fully disclose the research design.

The German translation of the Hospital Anxiety & Depression Scale (HADS) (Herrmann-Lingen et al., 2018, Zigmond and Snaith, 1983), was used to assess the severity of depressive and anxiety symptoms in order to control for their influence in the experimental manipulation of LTP-like cortical plasticity. The HADS is a self-rating instrument consisting

of each seven items for anxiety and depressive symptoms. Honarmand and Feinstein (2009) identified the HADS as a suitable instrument to assess the severity of depressive symptoms and symptoms of generalized anxiety in individuals with MS. Moreover, a review conducted by Bjelland et al. (2002) found the HADS to be a valid and highly reliable measure, reporting a mean Cronbach's alpha of 0.83 for the anxiety and 0.82 for the depression scale. All items were rated on a four-point Likert-Scale ranging from 0 to 3 with a total score ranging from 0 to 21 for both the depression and anxiety scale (Herrmann-Lingen et al., 2018, Zigmond and Snaith, 1983). According to the developers, scores ranging from 0 - 7 are considered normal, scores ranging from 8 - 10 are considered borderline abnormal and scores ranging from 11-21 are considered as clinically abnormal (Zigmond and Snaith, 1983, Herrmann-Lingen et al., 2018). An example of an item of the depression scale is: "I still enjoy the things I used to enjoy" (Herrmann-Lingen et al., 2018, Zigmond and Snaith, 1983). An example of an item of the anxiety scale is: "Worrying thoughts go through my mind" (Herrmann-Lingen et al., 2018, Zigmond and Snaith, 1983).

Symptoms of fatigue were assessed using the Fatigue Scale for Motor & Cognitive Functions (FSMC) (Penner et al., 2009). In that regard, the FSMC rather focusses on the susceptibility to fatigue over a period of time (trait fatigue), then on the acute experience of fatigue (state fatigue) (Penner, 2023, Malloy et al., 2021). The FSMC is a self-rating instrument consisting of each ten items for motor and cognitive fatigue. According to Penner et al. (2009), the FSMC is a valid and highly reliable instrument with an overall Cronbach's alpha of 0.91. All items are rated on a five-point Likert scale ranging from 1 (absolutely disagree) to 5 (absolutely agree), with a total score ranging from 10 to 50 for both, the motor and the cognitive scale (Penner et al., 2009). Thereby, a FSMC sum score of 20 - 42 indicates no fatigue, a score of 43 - 52 indicates mild fatigue, a score of 53 - 62 indicates moderate fatigue and a score of 63 - 100 indicates severe fatigue (Penner et al., 2009). An example of an item of the cognitive scale is: "Because of my exhaustion, it is harder for me to learn something new than it used to be" (Penner et al., 2009). An example of an item of the motor scale is: "My movements clearly slow down in a state of exhaustion" (Penner et al., 2009).

Information processing speed was assessed using the Rao-adapted version of the Symbol Digit Modalities Test (SDMT) (Rao, 1990), originally developed by Smith (1982). It is a reliable and valid measure of information processing speed in multiple sclerosis (Sonder et al., 2014, Benedict et al., 2008). According to the SDMT manual published by Smith (1982) the test procedure can be described as follows: in the SDMT nine different symbols are each paired with a digit from 1-9. These symbols are depicted in a random order in 8 rows on a sheet of paper (210 x 297 mm), with each row depicting 15 symbols. Under each symbol is a blank space, where the matching digit of the symbol can be filled in. On top of the sheet is a legend of the symbol-digit pairs. The first ten items are not counted and serve to familiarize with the test. Usually, the test is administered twice: manually and verbally (Smith, 1982). However, as it is common in MS research, the SDMT was only administered verbally to reduce the impact of motor disability on SDMT scores (Benedict et al., 2017). During verbal administration, 90 seconds of time are given to correctly report as many symbol-digit pairs as possible (Smith, 1982). Scoring involves the summation of the number of correct responses given in that time interval (Smith, 1982). Scores range from 0 - 110, with higher scores indicating higher information processing speed (Smith, 1982). These raw scores were converted into z-scores using the normative regression-based formula proposed by Scherer et al. (2004), which is based

on data of German speaking MS patients and healthy individuals. According to the authors, z-scores lower than, or equal to -1.68 are considered pathological (Scherer et al., 2004).

The Brief Visuospatial Memory Test - Revised (BVMT-R) was used to assess visuospatial short-term memory and learning (Benedict, 1997). The BVMT-R is a reliable and valid instrument in assessing visuospatial short-term memory (Benedict et al., 1996) and detecting cognitive deficits in MS (Filser et al., 2018). According to the manual provided by Benedict (1997), the test procedure can be described as follows: the BVMT-R consists of 6 different geometric figures, which are printed in a two-by-three array on a card (210 x 297 mm). In three trials, individuals are required to look at this card for ten seconds and memorize the design of the figures (size and form) as well as their position within the array. After that, as many of these six figures have to be drawn from memory in the correct design and position with a pencil on a blank sheet of paper (210 x 297 mm) without a time limit. During each trial, an eraser may be used for error correction. A maximum of two points can be earned for each correctly memorized figure: one point for the correct design and one point for the correct position within the array (Benedict, 1997). This results in a score range between 0 and 12 per trial (Benedict, 1997). The scores of all three trials are then summed to a total score, which can range between 0 and 36 (Benedict, 1997). According to the BVMT-R manual published by Benedict (1997), higher scores indicate higher visuospatial short-term memory and learning ability. Based on the norms provided by this manual, raw scores were converted into age adjusted z-scores (Benedict, 1997). Consistent with other research of our group, z-scores lower than, or equal to -1.68 were considered pathological to warrant comparability with the SDMT (Balloff et al., 2022). Other tests that were conducted as part of the longitudinal research design, but were not regarded in this cross-sectional study are the Parkinson Disease Questionnaire 39 (Berger et al., 1999), an adapted version of the cognitive leisure scale (Sumowski et al., 2010, Sumowski and Leavitt, 2013), and the Multiple Sclerosis Impact Scale 29 (Hobart et al., 2001).

2.3.7 Tests of Motor Function

In this research project, LTP-like plasticity was assessed by means of the increment in MEP amplitude of the right FDI after QPS. In that regard, previous research demonstrated associations between motor plasticity and motor functioning as reflected by manual dexterity and ambulation (Giffroy et al., 2017). Therefore, tools to assess manual dexterity and ambulation were included in this study.

The 9-Hole-Peg Test (NHPT) was used to measure manual dexterity (Mathiowetz et al., 1985, Kellor, 1971). The NHPT is a reliable and valid tool in the assessment of dexterity and upper limb motor functioning in individuals with MS (Mathiowetz et al., 1985, Hervault et al., 2017). Mathiowetz et al. (1985), Kellor (1971), describe the task as follows: first, the subject is seated at a table. On that table a wooden square box, containing nine wooden pegs is placed next to a wood block that contains nine empty holes, which are arranged in a three-by-three array. On a start signal, the NHPT requires the subject to place the nine pegs one by one and as quickly as possible in the nine holes of the wood block. Once, this is achieved, all pegs have to immediately be put back into the container in the same manner. The task is conducted sequentially in four trials with both the dominant and non-dominant hand being tested twice. The time to complete each trial is measured in seconds with a stopwatch. The average time is calculated for each hand separately, to account for asymmetry between hands (Mathiowetz et al., 1985). Overall, lager amounts of time needed indicate reduced manual dexterity

(Mathiowetz et al., 1985, Kellor, 1971). As this study solely focussed on the stimulation of the left M1, only the average times to complete the NHPT of the right hand were considered.

The 25 Foot Walk (T25FW), first introduced in the ambulatory index described by Hauser et al. (1983), was used to assess ambulation. It is a valid and reliable assessment tool to assess ambulation and lower limb motoric functioning in individuals with MS (Motl et al., 2017, Learmonth et al., 2012). Fischer et al. (1999) provide a standardized protocol of the T25FW, consisting of two trials. In the first trail, the T25FW requires the subject to walk a marked distance of 25 feet as quickly as possible with a steady and safe gait on a start command. In the second trail, the same task is repeated, with the subject being required to walk back the same distance. The time to walk each distance is measured from the start command in seconds with a stopwatch. For each individual, the average time was calculated for both trials, with larger amounts of time needed indicating impaired ambulation (Goldman et al., 2013). However, in line with previous research of our group, if a subject was not able to complete the T25FW due to disability, a substitute time based on a 90 percent confidence interval was calculated using the following formula: maximum time to complete the T25FW of the entire MS sample plus the standard deviation of the entire MS sample multiplied by 1.645 (Balloff et al., 2023b).

2.3.8 Demographic and Clinical Characteristics

In this study, age, sex, handedness, MS subtype, disability due to MS, disease duration, current intake of MS medication and the level of education were assessed as part of a structured interview. Thereby, the level of education was measured by counting the number of years spent in the educational system, by means of an assessment tool used in the standardization of the German version of the CERAD (Memory Clinic, n.d., Thalmann et al., 1998). The CERAD is a standardized and validated neuropsychological, neuropathological and clinical test battery to detect Alzheimer's disease (Morris et al., 1989, Aebi, 2002, Mirra et al., 1991). Previous research demonstrated a link, between cognitive ability and cortical plasticity (Burki et al., 2014) as well as between cognitive ability and the level of education as assessed by the CERAD (Luck et al., 2009). Thus, in this study, educational level was assessed in accordance with the CERAD.

The degree of disability due to MS was measured using the Expanded Disability Status Scale (EDSS) developed by Kurtzke (1983). The EDSS is a valid and commonly used instrument to quantify disability and monitor disease progression in MS (Meyer-Moock et al., 2014). Scores range on an ordinal scale from 0 (normal neurological status) to 10 (death due to MS) and increase in increment intervals of 0.5 once a score of 1 is reached (Kurtzke, 1983).

2.4 Experimental Procedure

Data acquisition was conducted in a single session. The assessment took place at the Brain Stimulation Laboratory at the UHD from May 2018 until October 2022 with the above-mentioned breaks. The procedure of the baseline and one-year follow up assessment used in this study is depicted in **Figure 3** and can be described as follows: Upon arrival at the laboratory, all participants were guided to an examination room and participants completed a questionnaire about their demographic characteristics and their medical history concerning MS. Next, a series of neuropsychological tests and tests of motor function were conducted by one of seven trained doctoral students from the faculty of medicine or one of two trained student research assistants from the faculty of psychology in the following order: The SMDT (Smith,

1982), the BVMT (Benedict, 1997), the 9-Hole-Peg-Test (Mathiowetz et al., 1985, Kellor, 1971) and the 25 Foot Walk (Hauser et al., 1983). Then, participants were requested to fill in self-report questionnaires in the following order: The Parkinson Disease Questionnaire 39 (Berger et al., 1999), the Work Ability Index (Tuomi et al., 1998), the Hospital Anxiety & Depression Scale (Zigmond & Snaith, 1983), the Fatigue Scale for Motor & Cognitive Functions (Penner et al., 2009), and the Multiple Sclerosis Impact Scale 29 (Hobart et al., 2001).

Thereafter, participants who were diagnosed with MS received a neurological examination. This examination was carried out by one of three certified neurologists of the UHD, who were involved in the study. The purpose of the examination was to interview participants about any medical related issues that would contraindicate TMS, to confirm the diagnosis of RRMS, SPMS or PPMS at baseline, to check for potential disease progress at follow-up and to estimate the participants EDSS at each time of assessment.

After that, the aforementioned students and student research assistants, who were also trained in performing the TMS protocol, guided participants to a room were TMS stimulation took place. There, participants were seated comfortably in a reclining armchair with both arms placed on armrests and electrodes were attached to the right FDI. Then, participants were instructed to keep the muscles of the target muscle relaxed during assessment and to only contract these muscles, when they were explicitly told to do so. Moreover, participants were instructed to move their body as little as possible, to minimize conversation during assessment and to keep count of the number of TMS pulses during assessment, in order to minimize MEP amplitude changes associated with fluctuations in attention (Noreika et al., 2020) and muscle contraction (Muellbacher et al., 2000). Yet, participants were told, that they were allowed to move their body and talk during the breaks of assessment, which were scheduled after each of the 7 stimulus blocks as well as after the QPS administration.

After these general instructions, the position of the left M1 was approximated with a measuring tape and marked with a pen. Then, the motor hotspot of the right FDI was determined from that point using monophasic TMS and an individual stimulus intensity for each participant. Once the motor hot-spot was identified, it was also marked and RMT, AMT, MEP 0.5 mV and MEP latency were assessed at that location in that order. Meanwhile, participants were instructed to keep the target muscle relaxed during the assessment of RMT and MEP 0.5 mV. Conversely, participants were instructed to keep their right FDI contracted at about 10-20% of their maximum force during the determination of their AMT and at about 30% of their maximum force during the assessment of their MEP latency. Subsequently, stimulus intensities for the assessment of MEP latency as well as rTMS and QPS were calculated for each participant. After that, the resulting stimulus intensities were entered into the Signal version 6.02 (Cambridge Electronic Design Ltd., Cambridge, UK) stimulation rTMS protocol and assessment was started.

The rTMS protocol consisted of three phases. In the first phase, the rTMS pre-QPS assessment was conducted. This was followed by a 5-minute break, in which the QPS was prepared. In the second phase, QPS was administered for 30 minutes to induce cortical plasticity (Matsumoto, & Ugawa, 2020). Another 5-minute break followed to prepare for rTMS post-QPS assessments. In the third phase, cortical plasticity was assessed through six consecutive rTMS post-QPS assessments, which were interspersed with 4-minute breaks. After that, participants were thanked for participation, received a free parking ticket and if applicable, 30 Euros as a reward.

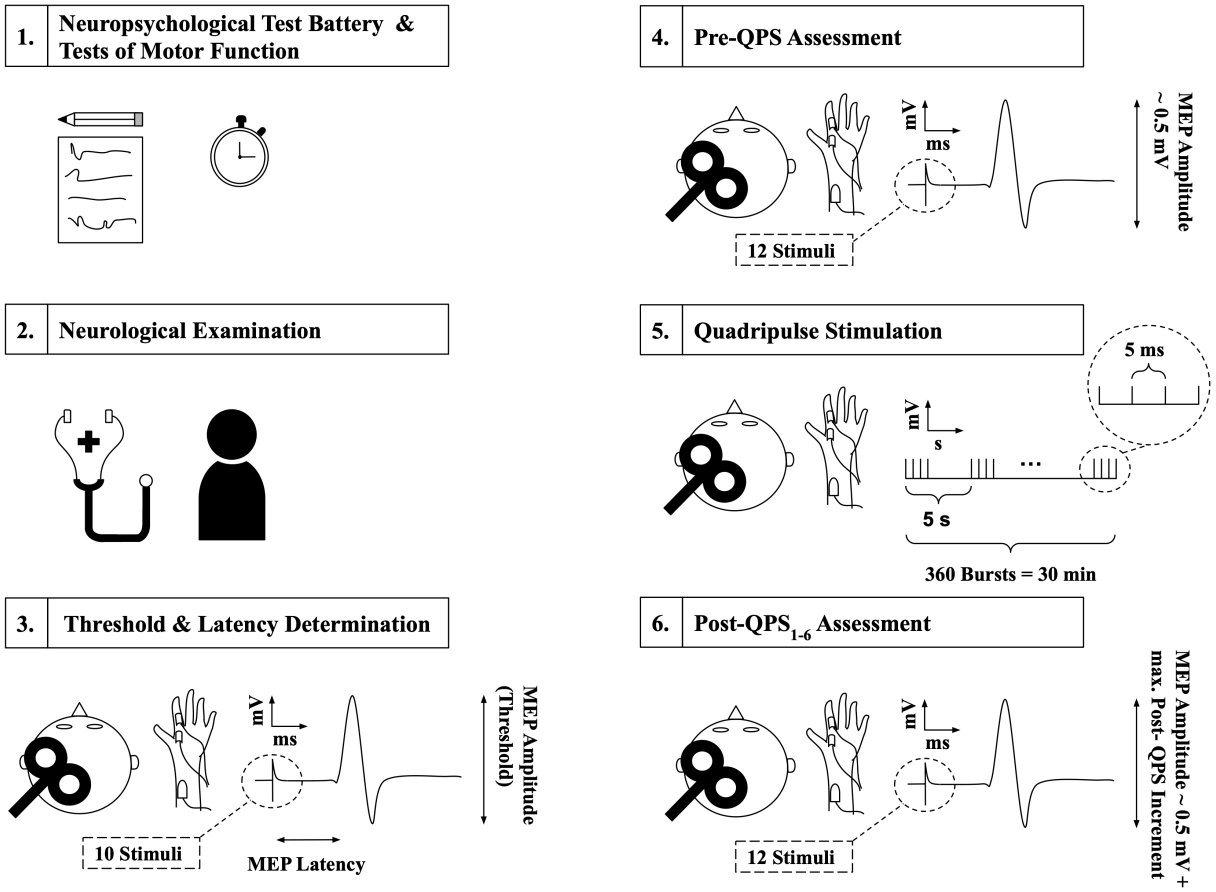

Fig. 3: Study Design.

This illustration depicts the study design. Firstly, participants completed a number of neuropsychological tests and tests of motor function, as well as questionnaires about their demographic characteristics and their medical history. Secondly, participants with MS received a neurological examination by a certified neurologist to confirm MS diagnoses and rule out any contraindications of participation. Thirdly, in preparation for the conduction of the rTMS protocol, thresholds for monophasic rTMS and QPS, as well as MEP latency were determined. For that purpose, each ten stimuli were applied and averaged. Fourthly, pre-QPS assessment was conducted to receive baseline values of excitation. Therefore, a block of six different stimulus intensities of each 12 stimuli with an ISI of 5 s were applied. The resulting peak to peak amplitudes were averaged for each stimulus intensity. In this study however, only the threshold eliciting an average MEP amplitude of approximately 0.5 mV was of interest. Fifthly, 360 QPS bursts with an IBI of 5 s and an ISI of 5 ms was applied over a period of 30 min to induce LTP-like cortical plasticity. Sixthly, six consecutive blocks (post-QPS_{1-6}) mirroring those of the pre-QPS assessment were conducted after QPS. Yet, for each participant, only the post-QPS block with the highest average MEP increment of the MEP 0.5 mV threshold (post-QPS_{max}) was of interest in this study. Abbreviations: IBI, interburst interval; ISI, interstimulus interval; max., maximum; MEP, motor evoked potential; mV, millivolts; ms, milliseconds; s, seconds. *Note*. Adapted from "Long-term potentiation-like plasticity is retained during relapse in patients with Multiple Sclerosis" by C. Balloff, S. Novello, A.-S. Stucke, L.K Janssen, E. Heinen, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner, P. Albrecht, S.J. Groiss, 2023, *Clinical Neurophysiology*, 155, p. 79. (https://doi.org/10.1016/j.clinph.2023.07.013). Copyright 2023 by Elsevier B.V..

2.5 Statistics

2.5.1 Software

For statistical analysis, raw data of the MEP amplitude and latency recordings were extracted from Signal version 6.02 (Cambridge Electronic Design Ltd., Cambridge, UK). Mean MEP latency, as well as mean MEP amplitudes for each stimulus intensity for each time point (Baseline and post-QPS_1 - post-QPS_6) were calculated in Microsoft Excel Version 16 (Microsoft Corporation®). All data were stored in an IBM SPSS Version 26 data frame (IBM

Corporation). However, the statistical analysis comprising descriptive statistics of the participant characteristics and multilevel mixed models were conducted using R Version 4.3.1 (R Core Team, 2023). Multilevel mixed modelling was conducted using the "nlme" package (Pinheiro et al., 2023). Results are reported based on the "anova.lme" and the "summary" function of the same package. Due to the slightly unbalanced design and missing values, analyses using Type III sum of squares was conducted (van Ginkel and Kroonenberg, 2021, Lewsey et al., 1997). Planned comparisons and post hoc contrasts were conducted using the "contrast" function of the "emmeans" package (Lenth, 2023).

2.5.2 Analyses of Participant Characteristics

Parametric and non-parametric tests were conducted to evaluate, whether participants' characteristics including demographic and clinical characteristics, neuropsychological test-scores, results of the tests of motor function, as well as electrophysiological measures including the number of excluded frames before and after intervention, were balanced across groups. In that regard, either Kruskal-Wallis test or Wilcoxon rank-sum test was conducted, when significant Shapiro Wilk test and/or Levene's test indicated that assumptions of one-way independent ANOVA or independent t-test were violated. If significant group differences were detected in one-way independent ANOVA or Kruskal-Wallis test, post-hoc contrasts were conducted using either independent t-test or Dunn's test.

In order to compare frequencies across groups, the chi-square test was conducted. However, when cell count is lower than five in at least one group, a chi-square distribution cannot be assumed (Pandis, 2016). Therefore, in these cases Fisher's exact test was conducted instead. Significant results of both tests were followed-up using paired Fisher's exact test. For all statistical analyses described in this section and in the sections below, significance values were set at $p < .05$. Moreover, to control for multiple comparisons, the p-values of analyses concerning the participant characteristics were Bonferroni adjusted (Dunn, 1961, Armstrong, 2014).

2.5.3 Multilevel Mixed Modelling

In this study, participants completed repeated measurements of their mean MEP amplitudes (pre-QPS and post-$QPS_{1\text{-}6}$ assessment), resulting in a nested data structure, in which measurements were nested within participants. To account for this nested data structure, a multilevel mixed model was specified in which measurements of the mean MEP amplitude constituted level-1 and participants level-2 data. As stated above, solely the increment of mean MEP amplitude from the pre-QPS assessment to the maximum mean MEP amplitude (post-QPS_{max}) of the six post-QPS assessments were of interest. Thus, mean pre-QPS and mean post-QPS_{max} MEP amplitudes formed the outcome variable. Thereby, time of assessment (pre-QPS vs. post-QPS_{max}) served as a level-1 predictor and group (HC, RRMS and PMS) as a level-2 predictor. In that connection, the basic model contained the interaction term between time of assessment and group (time*group) to identify differences in mean MEP amplitude and therefore, cognitive plasticity between individuals with PMS, RRMS and HC. To detect the hypothesized between group differences, the time*group interaction was followed up with planned comparisons using Hochberg's correction (Hochberg, 1988). This type I error correction was chosen, because it is suitable for unbalanced designs and provides good power (Kim, 2015, Blakesley et al., 2009). As the intercept was adjusted to approximately 0.5 mV, no random intercept was specified. Yet, a random slope was specified for time of assessment to

control for individual variability in the degree of cortical plasticity and response to QPS intervention. Furthermore, no specific variance or error structure was assumed. However, Akaike's information criterion (*AIC)* was used to exploratively evaluate whether the inclusion of any of these structures improved model fit (Akaike, 1987).

Because previous research demonstrated associations between rTMS induced increments in mean MEP amplitude and age, sex as well as mean MEP latency (Vallence et al., 2023, Pitcher et al., 2003), these variables and their interaction terms were added as level-2 covariates to the basic model. Moreover, to account for other potential confounding factors, demographic and clinical characteristics, neuropsychological test scores as well as TMS thresholds that showed significant group differences were added as exploratory level-2 covariates to the model. In that regard, to facilitate interpretability, all continuous predictors were centred on their mean. In terms of model selection, backward elimination was applied to exploratory covariates, because it reduces the neglection of suppressor effects compared to other selection procedures (Paulhus et al., 2004). After that, the inclusion or exclusion of the remaining variables to the final model was based on *AIC*, which was used to evaluate the goodness of fit of each more advanced model compared to the basic model (Akaike, 1987). Post hoc tests were conducted to follow-up significant main effects of covariates. The variance inflation factor (*VIF*) with a cut-off value of ≥ 5, indicating high correlation, was applied to test for multicollinearity (Kim, 2019). All model parameters were estimated with the restricted maximum likelihood approach, because it more accurately estimates standard errors of the fixed effects in small samples, than the maximum likelihood method (McNeish and Stapleton, 2016).

Finally, an exploratory analysis was conducted to control for systematic error resulting from the allocation of individuals with PPMS and SPMS to the same group. For that purpose, the PMS group was divided into a PPMS and SPMS group. After that, the same multilevel mixed model from the main analysis was refitted with a group variable containing four levels: PPMS, SPMS, RRMS and HC. Complementary to the main analysis, planned comparisons using Hochberg's correction were conducted to test for differences in the increment of mean MEP amplitude from pre to post-QPS_{max} assessment between participants with PPMS and SPMS, between participants with PPMS and RRMS, between participants with SPMS and RRMS, between participants with PPMS and HC and between participants with SPMS and HC.

2.5.4 Effect Size

Cohen's *d* (Cohen, 1988) was calculated to determine between-group effect sizes of the increment in mean MEP amplitude from pre- and post-QPS_{max} assessment. According to Cohen (1992) effect size is considered small when $d \leq 0.2$, medium when $d \geq 0.5$ but < 0.8 and large when $d \geq 0.8$. For the calculation of Cohens *d*, the pooled *SD* of the pre-QPS and post-QPS_{max} mean MEP amplitudes was used (Morris, 2008). Within-group effect sizes were computed by subtracting the mean MEP amplitude at pre-QPS assessment from the mean MEP amplitude at post-QPS_{max} for each of the groups separately and dividing the result by the pooled *SD* of the pre-QPS assessment (Morris, 2008):

$$\frac{(M_{\text{post-QPS}_{\text{max}},\,\text{HC}} - M_{\text{pre-QPS}})}{SD_{\text{pre-pooled}}}$$

Between-group effect sizes were computed for the comparisons between HC and individuals in the RRMS and PMS groups. This was done, by subtracting the difference in mean MEP amplitude of the RRMS group or the PMS group from the difference in mean MEP amplitude MEP amplitude of the HC (Morris, 2008):

$$\frac{(M_{\text{post-QPS}_{\text{max}},\ \text{HC}} - M_{\text{pre},\ \text{HC}}) - (M_{\text{post-QPS}_{\text{max}},\ \text{RRMS/PMS}} - M_{\text{pre},\ \text{RRMS/PMS}})}{SD_{\text{pooled}}}$$

In addition, between-group effect sizes were computed for the comparisons between individuals in the RRMS and PMS groups. This was done, by subtracting the difference in mean MEP amplitude of the PMS group from the difference in MEP amplitude of the RRMS group (Morris, 2008).

$$\frac{(M_{\text{post-OPS}_{\text{max}},\ \text{RRMS}} - M_{\text{pre},\ \text{RRMS}}) - (M_{\text{post-QPS}_{\text{max}},\ \text{PMS}} - M_{\text{pre},\ \text{PMS}})}{SD_{\text{pooled}}}$$

3. Results

3.1 Participant Characteristics

A summary of the results comparing the three groups in terms of their demographic and clinical characteristics, their neuropsychological test scores and motor function test results as well as their electrophysiological measures is illustrated in **Tables 1 – 3**. To warrant readability, only test-statistics and *p*-values of the significant post-hoc tests, which identify between group differences, are reported below. The test-statistic and *p*-values of significant and non-significant omnibus tests are solely disclosed in the aforementioned tables.

Concerning the demographic and clinical characteristics (**Table 1**), participants in the PMS group had significantly higher EDSS scores (*Mdn* = 5) than participants in the RRMS group (*Mdn* = 2), $W(2) = 178.5$, $p < .001$. This shows that participants with PMS had higher degrees of disability than participants with RRMS. In line with that finding, Fisher's exact test showed that participants in the PMS group had significantly higher unemployment rates than HC $p < .01$.

Concerning the neuropsychological test scores (**Table 2**), participants in the PMS group had significantly lower sum-scores (*Mdn* = 42.50) on the SDMT than people in the RRMS group (*Mdn* = 51.50), $z = -3.04$ $p = .007$ and HC (*Mdn* = 54.50) $z = -4.55$ $p < .001$. Concordantly, participants in the PMS group had significantly lower z-scores (*Mdn* = - 1.18) on the SDMT than participants in the RRMS group (*Mdn* = - 0.06), $z = -3.00$ $p = .008$ and HC (*Mdn* = 0.43) $z = -4.87$, $p < .001$. This indicates, that participants in the PMS group had lower degrees of information processing speed than both, participants with RRMS and HC. Regarding the BVMT, participants in the PMS group had lower sum-scores (*Mdn* = 19.00), $z = -4.21$, $p < .001$, and *z*-scores (*Mdn* = -1.19), $z = -4.40$, $p < .001$, than HC (*Mdn* = 28.00; *Mdn* = 0.81), meaning that they had lower degrees of visuospatial short-term memory and learning than healthy participants. In line with that finding, Fisher's exact test showed that BVMT scores that are classified as pathological, were more frequent in participants with PMS than HC $p < .02$. Concerning the HADS, participants in the RRMS group had higher sum-scores on the anxiety scale (*Mdn* = 7.00), $z = 3.51$, $p = .001$, than HC (*Mdn* = 3.00). More precisely, Fisher's exact test demonstrated that compared to HC, participants in the RRMS group more frequently

showed borderline abnormal $p = .045$ and clinical abnormal scores $p = .032$. This indicates that participants in the RRMS group demonstrated more symptoms of anxiety than HC. Moreover, participants in the PMS (*Mdn* = 4.00), $z = 4.35$, $p < .001$ and RRMS group (*Mdn* = 5.00), $z = 4.67$, $p < .001$, had significantly higher sum-scores on the HADS depression scale than HC (*Mdn* = 1.00). In that regard, Fisher's exact test demonstrated that compared to HC, participants in the PMS group showed significantly more often scores that were borderline abnormal $p = .032$. That means that participants in the PMS group demonstrated more severe symptoms of depression than HC. Lastly, participants in the PMS (*Mdn* = 70.50), $z = 5.84$, $p < .001$, and RRMS group (*Mdn* = 65.17), $z = 4.35$, $p < .001$, had significantly higher FSMC sum-scores than HC (*Mdn* = 28.00). Paired Fisher's exact tests revealed that compared to HC, participants in the PMS $p < .001$ and RRMS $p < .001$ group reported significantly more often severe fatigue than no fatigue. Furthermore, paired Fisher's exact tests revealed that compared to HC, participants in the PMS $p = .006$ and RRMS $p = .046$ group also reported significantly more often severe fatigue than mild fatigue. Thus, these findings indicate that participants in the PMS and RRMS group demonstrated more severe symptoms of fatigue than HC.

Concerning the results of the tests of motor function (**Table 2**), participants in the PMS group took significantly more time to absolve the T25FW (*Mdn* = 6.39) than participants in the RRMS group (*Mdn* = 4.46), $z = 3.30$, $p = .003$ and HC (*Mdn* = 3.54), $z = 6.59$, $p < .001$. Similarly, participants in the RMS group took significantly more time to absolve the T25FW than HC, $z = 3.33$, $p = .003$. This demonstrates, that both, participants in the PMS and RRMS group had impaired ambulation compared to HC. Furthermore, it can be deduced that compared to participants in the RRMS group, ambulation was more impaired in participants with PMS. In regard to the NHPT, participants in the PMS group (*Mdn* = 25.26), $z = 5.77$, $p < .001$ and participants in the RRMS group (*Mdn* = 22.06), $z = 3.68$, $p < .001$ took significantly more time to complete the task than HC (*Mdn* = 18.88), indicating that participants in the PMS and RRMS group had impaired manual dexterity compared to HC.

Concerning the electrophysiological measures (**Table 3**), participants in the PMS group had significantly higher RMT (*Mdn* = 55.50), $z = 3.15$, $p = .005$, AMT (*Mdn* = 45.50), $z = 3.17$, $p = .005$, and MEP 0,5 mV thresholds (*Mdn* = 81.00), $z = 4.38$, $p < .001$, than HC (*Mdn* = 48.00; *Mdn* = 39.00; *Mdn* = 58.00), indicating that they needed higher stimulation intensities to achieve the same MEP response. In addition, participants in the PMS group had on average significantly lower MEP amplitudes at pre-QPS assessment (*M* = 0.48) than HC (*M* = 0.57), $t(61.3) = 2.61$ $p = .033$. This shows, that compared to HC, cortical excitability before QPS intervention was lower in the PMS group. Moreover, participants in the PMS group (*Mdn* = 25.39) had significantly longer durations of mean MEP latency than participants in the RRMS group (*Mdn* = 23.40), $z = 2.73$, $p = 0.019$, and HC (*Mdn* = 22.77), $z = 4.03$, $p < .001$. Furthermore, paired Fisher's exact tests revealed that individuals with PMS had significantly more often mean MEP latencies that are considered pathological than participants with RRMS, $p < .001$ and HC, $p < .001$. Compared to HC (*Mdn* = 25.04), pathological latencies were significantly longer in the PMS group (*Mdn* = 29.43), $z = 2.68$, $p < .022$. This means, that participants with PMS had worse MEP latencies than participants with RRMS or HC. Lastly, significantly more MEP frames were excluded in the PMS group during pre-QPS and post-QPS assessment (*Mdn* = 3.50), $z = 2.69$, $p = 0.021$, compared to the RRMS group (*Mdn* = 3.00). This shows that during both times of assessment, MEP frames in the PMS group were more often contaminated by voluntary muscle movement, or other measurement artefacts, than MEP frames in the RRMS group.

Table 1: Demographic and Clinical Characteristics by Group.

Characteristics	Group			Test Statistic	*p*
	PMS (*N* = 34)	RRMS (*N* = 30)	HC (*N* = 30)		
	Mean (*SD*) Median [*IQR*] *n* {%}	Mean (*SD*) Median [*IQR*] *n* {%}	Mean (*SD*) Median [*IQR*] *n* {%}		
Age[a]	50.53 (8.17)	47.80 (7.03)	50.73 (9.58)	$F(2, 91) = 1.18$	.311
Education[a]	15.00 [5.00]	16.00 [5.50]	17 .00 [4.75]	$H(2) = 3.15$	.207
Disease Duration[a]	12.19 [15.62]	13.45 [9.57]	-	$W(2) = 523.00$	.861
EDSS	5 [3.00]	2 [1.88]	-	$W(2) = 178.50$	< .001
Sex:					
man	18 {52.94}	14 {46.67}	14 {46.67}		
women	16 {47.06}	16 {53.33}	16 {53.33}	$\chi^2(2, 94) = 0.34$	.843
Handedness[b]**:**					
right-handed	32 {94.12}	27 {90.00}	27 {90.00}		
left-handed	1 {2.94}	3 {10.00}	3 {10.00}	-	.554
Employment:					
yes	16** {47.06}	23 {76.67}	26** {86.67}		
no	18** {52.94}	7 {23.33}	4** {13.33}	-	.002
MS Medication:					
yes	28 {82.35}	25 {83.33}	-		
no	6 {17.65}	5 {16.67}	-	$\chi^2(1, 64) = 0.04$	.845

Note. Standard deviations are between parentheses. Interquartile ranges are between squared brackets. Percentages are between curly brackets and may, due to rounding and missing values, not add up to 100. As assumptions for parametric testing were violated, Kruskal-Wallis tests were conducted. Fisher's exact test was conducted instead of Chi-square test, when cell count was lower than five. Significant omnibus tests were followed up with Bonferroni corrected post-hoc test (Dunn's test or paired Fishers exact test). Asterisks, daggers and double daggers reflect significant post-hoc group differences. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 06. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

[a] Measured in years.

[b] Missing value: $N = 1$ (PMS).

HC vs. PMS: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$; HC vs. RRMS: $^{\dagger}p < .05$, $^{\dagger\dagger}p < .01$, $^{\dagger\dagger\dagger}p < .001$;
RRMS vs. PMS: $^{\ddagger}p < .05$, $^{\ddagger\ddagger}p < .01$, $^{\ddagger\ddagger\ddagger}p < .001$

Table 2: Results of the Neuropsychological Test Battery and Tests of Motor Function by Group.

Tests Results	Group			Test Statistic	*p*
	PMS (*N* = 34)	RRMS (*N* = 30)	HC (*N* = 30)		
	Median [*IQR*] *n* {%}	Median [*IQR*] *n* {%}	Median [*IQR*] *n* {%}		
BVMT:					
sum-score	19.00*** [12.75]	23.50 [12.00]	28*** [5.50]	$H(2) = 17.96$	< .001
z-score	-1.19*** [2.14]	- 0.14 [2.37]	0.81*** [1.12]	$H(2) = 19.42$	< .001
normal	22* {64.71}	23 {76.67}	28* {93.33}		
pathological	12* {35.29}	7 {23.33}	2* {6.67}	-	.018
SDMT:					
sum-score	42.50*** ‡‡ [13.50]	51.50‡‡ [22.50]	54.50*** [18.50]	$H(2) = 21.73$	< .001
z-score	-1.18*** ‡‡ [1.17]	-0.06‡‡ [1.84]	0.43*** [1.91]	$H(2) = 24.41$	< .001
normal	26 {76.47}	25 {83.33}	28 {93.33}		
pathological	8 {23.53}	5 {16.67}	2 {6.67}	-	.200
FSMC:					
sum-score	70.50*** [33.75]	65.17††† [30.00]	28.00*** ††† [23.50]	$H(2) = 36.52$	< .001
no fatigue	5*** {14.71}	6††† {20.00}	21*** ††† {70.00}		
mild fatigue	3** {8.82}	5† {16.67}	5** † {16.67}	-	< .001
moderate fatigue	5 {14.71}	3 {10.00}	3 {10.00}		
severe fatigue	21*** ** {61.76}	16††† †{53.33}	1*** ††† ** † {3.33}		
HADS-A:					
sum-score	4.50 [5.50]	7.00†† [6.00]	3.00†† [3.50]	$H(2) = 12.32$	.002
normal	25 {73.53}	16† † {53.33}	28† † {93.33}		
borderline abnormal	8 {23.53}	9† {30.00}	2† {6.67}	-	.004
clinically abnormal	1 {2.94}	5† {16.67}	0† {0.00}		
HADS-D:					
sum-score	4.00*** [7.00]	5.00††† [4.00]	1.00*** ††† [3.00]	$H(2) = 26.92$	< .001
normal	23* {67.65}	23 {76.67}	30* {100.00}		
borderline abnormal	6* {17.65}	3 {10.00}	0* {0.00}	-	.007
clinically abnormal	5 {14.71}	4 {13.33}	0 {0.00}		
9-Hole-Peg-Test[a b c]	25.26*** [9.01]	22.06††† [5.02]	18.88*** ††† [2.09]	$H(2) = 33.82$	< .001
25 Foot Walk[a b d]	6.39*** ‡‡ [3.85]	4.46†† ‡‡ [1.59]	3.54*** †† [0.94]	$H(2) = 43.43$	< .001

Note. Interquartile ranges are between squared brackets. Percentages are between curly brackets and may, due to rounding, not add up to 100. As assumptions for parametric testing were violated, Kruskal-Wallis tests were conducted. Fisher's exact test was conducted because each variable had at least one cell count lower than five. Significant omnibus tests were followed up with Bonferroni corrected post-hoc tests (Dunn's test or paired Fishers exact test). Asterisks, daggers and double daggers reflect significant post-hoc group differences. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 06. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

[a] Measured in seconds.

[b] Missing value: $N = 5$ ($n = 2$ PMS; $n = 3$ HC).

[c] Only results of the right hand are displayed.

[d] $N = 8$ ($n = 4$ PMS; $n = 4$ RRMS) were assigned a substitute value because they could not absolve the task.

HC vs. PMS: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$; HC vs. RRMS: $^{†}p < .05$, $^{††}p < .01$, $^{†††}p < .001$;

RRMS vs. PMS: $^{‡}p < .05$, $^{‡‡}p < .01$, $^{‡‡‡}p < .001$

Table 3: Electrophysiological Measures by Group.

Electrophysiological Measures	Group			Test Statistic	*p*
	PMS (*N* = 34)	RRMS (*N* = 30)	HC (*N* = 30)		
	Mean (*SD*) Median [*IQR*] *n* {%}	Mean (*SD*) Median [*IQR*] *n* {%}	Mean (*SD*) Median [*IQR*] *n* {%}		
Thresholds:					
RMT[a]	55.50** [14.00]	51.50 [18.00]	48.00** [6.75]	$H(2) = 10.00$	.007
AMT[a]	45.50** [13.00]	44.50 [11.00]	39.00** [5.00]	$H(2) = 1.18$	.005
MEP 0,5 mV[a]	81.00*** [34.00]	66.50 [30.00]	58.00*** [11.25]	$H(2) = 19.24$	< .001
MEP Amplitude:					
Pre-QPS[b c]	0.48* (0.14)	0.52 (0.13)	0.57* (0.13)	$F(2, 91) = 3.42$	.037
Post-QPS_{max}[b c]	0.67 [0.56]	0.96 [0.76]	0.96 [0.72]	$H(2) = 5.50$	.064
Latency[d e]:	25.39***‡ [5.96]	23.40‡ [2.07]	22.77*** [2.77]	$H(2) = 17.14$	< .001
normal[d f]	23.31[1.68]	22.99 [1.72]	22.5 [1.73]	$H(2) = 2.46$	.293
pathological[d g]	29.43* [4.15]	26.47 [4.09]	25.04* [0.18]	$H(2) = 7.17$	.028
normal[h f]	13**‡ {38.24}	22‡ {73.33}	23** {76.67}		
pathological[h g]	19**‡ {55.88}	7‡ {23.33}	5** {16.67}	$\chi^2(2, 94) = 13.51$	.001
Excluded Frames	3.50‡ [4.75]	3.00‡ [2.00]	3.50 [3.75]	$H(2) = 7.37$	.025

Note. Interquartile ranges are between squared brackets. Percentages are between curly brackets and may, due to rounding and missing values, not add up to 100. Because assumptions for parametric testing were violated, non-parametric Kruskal-Wallis tests were conducted. Significant omnibus tests were followed up with Bonferroni corrected post-hoc test (Dunn's test or paired Fishers exact test). Asterisks, daggers and double daggers reflect significant post-hoc group differences. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 06. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

[a] Measured in percentages.

[b] MEP Amplitude measured in mV.

[c] MEP amplitude at a stimulation intensity of the MEP 0,5 mV threshold.

[d] Measured in milliseconds.

[e] Missing value: $N = 5$ ($n = 2$ PMS; $n = 1$ RRMS; $n = 2$ HC).

[f] Latencies < 24.5 ms

[g] Latencies > 24.5 ms

[h] measured as frequency

HC vs. PMS: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$; HC vs. RRMS: $^{†}p < .05$, $^{††}p < .01$, $^{†††}p < .001$;
RRMS vs. PMS: $^{‡}p < .05$, $^{‡‡}p < .01$, $^{‡‡‡}p < .001$

3.2 Multilevel Mixed Modelling

3.2.1 Model Selection

In addition to the three predefined covariates age, sex and MEP latency, the variables RMT threshold, number of excluded MEP frames, EDSS scores, employment rate, sum-scores of the BVMT, SDMT, FSMC, HADS-A and HADS-D, as well as time to absolve the NHPT and T25FW, were due to significant post-hoc between group differences, added as exploratory covariates. Although significant group differences were also found for AMT and MEP 0.5 mV thresholds, they were not included as exploratory covariates. The reason for this was that statistically significant high magnitude Spearman's rank correlations were found between AMT and RMT thresholds, $r_s(92) = .88$, $p < .001$, as well as MEP 0.5 mV and RMT thresholds, $r_s(92) = .81$, $p < .001$. Hence, the inclusion of all three variables to multilevel mixed model would

violate the assumption of independent predictors. To tackle this problem, only RMT threshold was assigned as an exploratory covariate, because it represents the minimum excitation threshold of the left motor cortex on which both, AMT and MEP 0.5 mV threshold depend (Ma et al., 2023). Likewise, only BVMT and SDMT sum-scores were included as exploratory covariates, because they are in comparison easier to interpret and due to matching of participants score dependency on age and education can be neglected.

Based on backward elimination of the exploratory covariates and *AIC* as a model fitting criterion, the basic model that included the covariates latency and age, as well as their interaction term, represented the data best. Moreover, *AIC* showed that the inclusion of a random slope for the variable "time of assessment", but not a random intercept improved model fit. In addition, *AIC* indicated that the imposition of any specific variance structure or autocorrelation did not improve model fit. In the following section, only the results of this final model are reported.

3.2.2 Main Analysis

Observed and predicted means of the final model are illustrated in **Table 4**. The parameter information of the fixed and random effects of the final model are summarized in **Table 5**, while ANOVA results of the final model are solely described in the text below. Moreover, mean MEP amplitude of the three groups (PMS, RRMS and HC) at pre- and post-QPS_{max} assessment are illustrated in **Figure 4**, while mean MEP amplitude of the three groups at pre- and post-QPS_{1-6} assessment are illustrated in **Figure 5**.

Overall, the fixed effects of the model explained 32% of the data, marginal R^2 = .32, while the fixed and random effects combined explained about 97% of the data, conditional R^2 = .97. Concerning the fixed effects, there was a significant intercept, $F(1, 86) = 452.69$, $p < .001$, indicating that MEP amplitude at pre-QPS assessment was significantly different from zero. In this respect, the regression coefficient of $b = .54$, indicated that the manipulation of identifying a stimulation intensity, evoking a mean MEP amplitude of about 0.5 mV at pre-QPS assessment was, on average, successful, $t(86) = 21.30$, $p < .001$. Moreover, in line with the hypothesis, there was a significant main effect of time of assessment on mean MEP amplitude $F(1, 86) = 32.86$, $p < .001$, indicating that mean MEP amplitudes between pre-QPS and post-QPS_{max} assessment were significantly different. Indeed, the model revealed that the time of assessment significantly predicts an increase in MEP amplitude from pre-QPS to post-QPS_{max} assessment, $b = 0.51$, $t(86) = 5.73$, $p < .001$. Regarding the random effects, MEP amplitude varied more strongly at post-QPS assessment, $Var_{post\text{-}QPSmax} = 0.26$, $SD = 0.51$, compared to pre-QPS assessment, $Var_{pre\text{-}QPS} = 0.01$, $SD = 0.10$. This high variability of MEP amplitudes is also reflected by the increase in error bars from pre-QPS to post-QPS assessment in **Figure 4** and **Figure 5**.

However, contrary to what was hypothesized, there was no significant main effect of group on MEP amplitude $F(2, 83) = 0.56$, $p = .576$, showing that across time points, mean MEP amplitude did, on average, not significantly differ between participants with PMS, RRMS and HC. In line with these findings, the model revealed that neither PMS, $b = - 0.04$, $t(83) = - 1.02$, $p = .310$ nor RRMS $b = - 0.03$, $t(83) = - 0.75$, $p = .454$ significantly predicted mean MEP amplitudes that were different from those predicted by HC. Moreover, contrary to what was hypothesized, there was no significant interaction effect between time of assessment and group on mean MEP amplitude $F(2, 83) = 0.71$, $p = .497$. This means that the increment in mean MEP amplitude from pre- to post-QPS_{max} assessment did not differ between participants with PMS,

RRMS and HC. More precisely, planned contrasts using Hochberg's correction revealed that the increment in MEP amplitude from pre- to post-QPS_{max} assessment did neither differ between participants with PMS and HC $t(86) = -0.91$, $p = .734$, $d = 0.41$, nor between participants with RRMS and HC $t(86) = 0.18$, $p = .857$, $d = 0.00$, nor between participants with RRMS and PMS $t(86) = -1.10$, $p = .734$, $d = 0.41$. Moreover, the model demonstrated that neither PMS, $b = -0.11$, $t(86) = -0.91$, $p = .367$ nor RRMS $b = -0.02$, $t(86) = 0.18$, $p = .857$ did significantly predict increments in mean MEP amplitude from pre- to post-QPS_{max} assessment that are significantly different from those predicted by HC. The non-significance of these findings is also reflected by the large overlapping error bars depicted in **Figure 4** and **Figure 5**.

However, contrasting with the statistical results, these graphs show non-significant trends, which suggests that individuals with PMS may have lower increments in post-QPS_{max} MEP amplitude than individuals with RRMS and HC (**Figure 4**). This non-significant trend is relatively consistent throughout the six post-QPS assessments (**Figure 5**). Yet, in support of the analysis the graphical trends depicted in **Figure 4** demonstrate that the increments in MEP amplitude of individuals with RRMS and HC are of a similar magnitude. Nevertheless, some small non-significant differences between HC and individuals with RRMS can be observed across the six time points (**Figure 5**). These non-significant trends might suggest that individuals with RRMS have lower increments in MEP amplitudes in the first half of assessment, when compared to HC, but higher MEP amplitudes in the second half of the assessment.

Regarding the covariates, there was a significant main effect of mean MEP latency on mean MEP amplitude, $F(1, 83) = 5.98$, $p = .017$. This indicates that across time points and groups, mean MEP amplitude was different for participants with different mean MEP latencies. In that regard, the model revealed that mean MEP latencies negatively predict mean MEP amplitudes $b = -0.04$, $t(83) = -2.45$, $p = .017$. Due to the mean centering of covariates, this means that participants with longer than average mean MEP latencies had lower mean MEP amplitudes than participants with average MEP latencies.

Regarding the covariate age, there was no significant main effect on mean MEP amplitude, $F(1, 83) = 0.59$, $p = .445$. This indicates that across time points and groups, mean MEP amplitude did not differ between participants of different ages. In line with that finding, the model revealed that differences in participants age did not significantly predict differences in mean MEP amplitude across time points $b = -0.01$, $t(83) = -0.77$, $p = .445$. Due to mean centering, this indicates that compared to participants who were of average age ($M = 49{,}69$), participants who were older or younger than average, did not have different mean MEP amplitudes across time. Furthermore, there was no significant interaction effect between mean MEP latency and age on MEP amplitude across groups and time points, $F(1, 83) = 3.69$, $p = .058$. Thus, across time points and groups, the influence of participant's mean MEP latency on mean MEP amplitude did not depend on participant's age. In accordance with that finding, the model showed that the magnitude of the influence of mean MEP latency on mean MEP amplitude was not significantly predicted by the age of participants, $b = 0.03$, $t(83) = 1.92$, $p = .058$.

Table 4: Observed and Predicted Means of the Final Multilevel Mixed Model.

Observed and model predicted means of MEP amplitude by group.

MEP Amplitude (mV)	Group					
	PMS (*n* = 34)		RRMS (*n* = 30)		HC (*n* = 30)	
	Mean (*SE*)	[95% CI]	Mean (*SE*)	[95% CI]	Mean (*SE*)	[95% CI]
Observed:						
Pre-QPS[a]	0.48 (0.02) {1.23}	-	0.52 (0.02) {1.65}	-	0.57 (0.02) {1.65}	-
Post-QPS_{max}[a]	0.87 (0.09)	-	1.04 (0.10)	-	1.09 (0.09)	-
Predicted:						
Pre-QPS[a]	0.50 (0.02)	[0.46 ; 0.55]	0.52 (0.02)	[0.47 ; 0.56]	0.54 (0.03)	[0.49 ; 0.59]
Post-QPS_{max}[a]	0.91 (0.09)	[0.72 ; 1.09]	1.05 (0.10)	[0.86 ; 1.24]	1.05 (0.10)	[0.86 ; 1.25]

Note. Standard errors are between brackets. 95% confidence intervals are between squared brackets. Effect sizes (Cohen`s *d*) of within group differences are between curly brackets. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 06. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

[a] MEP amplitude at a stimulation intensity of the MEP 0,5 mV threshold.

Table 5: Parameter Information of the Final Multilevel Mixed Model.

Parameter information for fixed and random effects of the final multilevel mixed model, comparing the maximum MEP amplitude after QPS with the MEP amplitude before QPS by group.

Parameter Information	Fixed Effects				Random Effects	
	β-coefficient (*SE*)	[95% CI]	Test Statistic	*p*	Variance	*SD*
Intercept	**0.54** (0.03)	[0.49 ; 0.59]	*t*(86) = 21.30	**< .001**	-	-
Time of Assessment:						
Pre-QPS[a b]	-	-	-	-	-	-
Post-QPS_{max}[b]	**0.51** (0.09)	[0.33 ; 0.69]	*t*(86) = 5.73	**< .001**	-	-
Group:						
HC[a]	-	-	-	-	-	-
RRMS	- 0.03 (0.03)	[- 0.09 ; 0.04]	*t*(83) = - 0.75	.454	-	-
PMS	- 0.04 (0.04)	[- 0.11 ; 0.04]	*t*(83) = - 1.02	.310	-	-
Covariates:						
Latency	**- 0.04** (0.02)	[- 0.07 ; - 0.01]	*t*(83) = - 2.45	**.017**	-	-
Age	- 0.01 (0.01)	[- 0.04 ; 0.02]	*t*(83) = - 0.77	.445	-	-
Interaction Terms:						
Post-QPS_{max}*RRMS	- 0.02 (0.13)	[- 0.23 ; 0.27]	*t*(86) = 0.18	.857	-	-
Post-QPS_{max}*PMS	- 0.11 (0.12)	[- 0.35 ; 0.13]	*t*(86) = - 0.91	.367	-	-
Latency*Age	0.03 (0.02)	[0.00 ; 0.06]	*t*(83) = 1.92	.058	-	-
Subject*Pre-QPS	-	-	-	-	0.01	0.10
Subject*Post-QPS_{max}	-	-	-	-	0.26	0.51
Residual	-	-	-	-	0.01	0.08

Note. Standard errors are between brackets. 95% confidence intervals of *β*-coefficients are between squared brackets and were estimated using bootstrapping method. Significant *β*-coefficients and *p*-values are highlighted in bold font. Conditional R^2 = .97. Marginal R^2 = .32. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 07. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

[a] Factor-level served as a reference group.

[b] MEP amplitude at a stimulation intensity of the MEP 0,5 mV threshold.

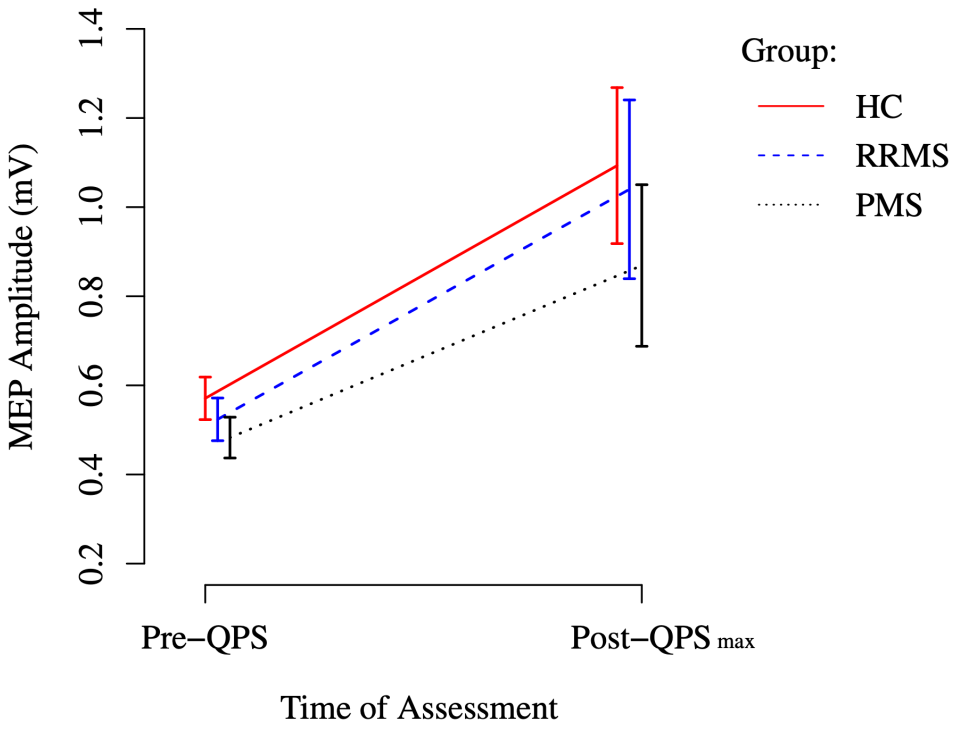

Fig. 4: MEP Amplitude from Pre-QPS to Post-QPS$_{max}$ Assessment by Group.
The figure illustrates the QPS-induced increment in mean MEP amplitude in mV from pre- to post-QPS$_{max}$ assessment of HC, as well as participants with RRMS and PMS. The lines of the graph representing the RRMS and PMS group were slightly shifted to the right so that error bars and their overlap can better be identified. Abbreviation: max, maximum; mV, millivolts. *Note*. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 08. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

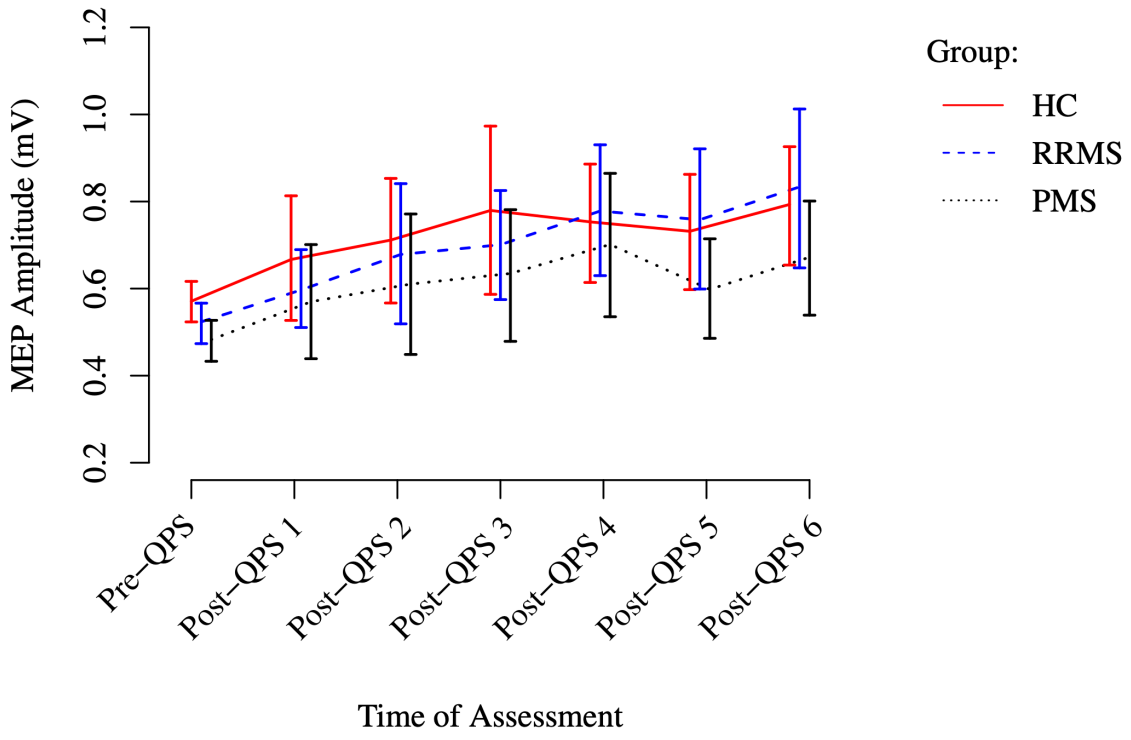

Fig. 5: MEP Amplitude across all Time points of Assessment by Group.
The figure illustrates the QPS-induced increment in mean MEP amplitude in mV from pre QPS assessment across all post-QPS assessments (post-QPS$_{1\text{-}6}$) of HC, as well as participants with RRMS and PMS. The lines of the graph representing the RRMS and PMS group were slightly shifted to the right so that error bars and their overlap can better be identified. Abbreviation: mV, millivolts. *Note*. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 08. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

3.2.3 Post Hoc Testing of the Main Analysis

Due to the significant main effect of mean MEP latency on mean MEP amplitude, a post-hoc analysis was conducted to test whether there was a significant interaction effect between latency and time of assessment (pre vs. post QPS). For that purpose, the final model was refitted including the time by MEP latency interaction term. The observed fixed and random effects of this refitted model are summarized in **Table 6**.

Overall, it was found that the refitted model fitted the data better AIC = 0.8 than the original model AIC = 17.1. In line with that finding, the fixed effects of the refitted model explained more variance of the data, marginal R^2 = .45. Indeed, there was a significant interaction effect between time of assessment and mean MEP latencies on mean MEP amplitude $F(1, 85) = 19.33$, $p < .001$. In comparison to the previous model, the statistical significance of the other fixed effects remained unchanged. Regarding the interaction effect, the refitted model demonstrated that mean MEP latency negatively predicts increments in mean MEP amplitude from pre- to post-QPS$_{max}$ assessment b = - 0.06, $t(85)$ = -4.40, $p < .001$. This means that compared to participants with average durations of mean MEP latencies, participants with longer than average mean MEP latencies had lower increments in mean MEP amplitudes from pre- to post-QPS assessment. The time of assessment (pre- and post-QPS$_{max}$) by MEP latency is illustrated in **Figure 6**, while the time of assessment (pre- and post-QPS$_{1\text{-}6}$) by MEP latency

is illustrated in **Figure 7**. To simplify interpretation, the continuous variable mean MEP latency was transformed into a categorical variable with two levels: a normal MEP latency (< 24.5 ms) and a pathological MEP latency group (> 24.5 ms). In accordance with the findings of the multilevel model, the depicted graph in **Figure 6** shows that individuals with pathological MEP latencies show a lower increase in MEP amplitude from pre-QPS to post-QPS_{max} assessment than individuals with normal MEP latencies. In addition, the depicted graph in **Figure 7** demonstrates that individuals with pathological MEP latencies show a lower increase in MEP amplitude throughout all six post-QPS assessments.

Table 6: Parameter Information of the Post Hoc Multilevel Mixed Model

Parameter information for fixed and random effects of the post hoc multilevel mixed model, comparing the maximum MEP amplitude after QPS with the MEP amplitude before QPS by group, including the time by latency interaction.

Parameter Information	**Fixed Effects**				**Random Effects**	
	***β*-coefficient (*SE*)**	**[95% CI]**	**Test Statistic**	***p***	**Variance**	***SD***
Intercept	**0.54** (0.03)	[0.49 ; 0.59]	$t(85) = 21.10$	**< .001**	-	-
Time of Assessment:						
Pre-QPS[a b]	-	-	-	-	-	-
Post-QPS_{max}[b]	**0.41** (0.08)	[0.24 ; 0.58]	$t(85) = 4.86$	**< .001**	-	-
Group:						
HC[a]	-	-	-	-	-	-
RRMS	- 0.02 (0.03)	[- 0.09 ; 0.05]	$t(83) = - 0.64$	.525	-	-
PMS	- 0.03 (0.04)	[- 0.10 ; 0.05]	$t(83) = - 0.70$	.486	-	-
Covariates:						
Latency[d]	**- 0.01** (0.00)	[- 0.02 ; - 0.01]	$t(83) = - 3.14$	**.002**	-	-
Age	- 0.001 (0.002)	[- 0.010 ; 0.002]	$t(83) = - 0.77$	.445	-	-
Interaction Terms:						
Post-QPS_{max}*RRMS	0.10 (0.13)	[- 0.13 ; 0.33]	$t(85) = 0.84$	.403	-	-
Post-QPS_{max}*PMS	0.11 (0.12)	[- 0.14 ; 0.35]	$t(85) = 0.88$	.383	-	-
Latency*Age[c d]	0.001 (0.000)	[0.000 ; 0.002]	$t(83) = 1.92$	.058	-	-
Post-QPS*Latency	**- 0.1** (0.01)	[- 0.10 ; - 0.03]	$t(85) = - 4.40$	**< .001**		
Subject*Pre-QPS	-	-	-	-	0.01	0.10
Subject*Post-QPS_{max}	-	-	-	-	0.21	0.50
Residual	-	-	-	-	0.01	0.08

Note. Standard errors are between brackets. 95% confidence intervals of *β*-coefficients are between squared brackets and were estimated using bootstrapping method. Significant *β*-coefficients and *p*-values are highlighted in bold font. Conditional R^2 = .97. Marginal R^2 = .32. *Note*. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 07. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

[a] Factor-level served as a reference group.

[b] MEP amplitude at a stimulation intensity of the MEP 0,5 mV threshold.

[c] Due to the small size of the estimated effect of the covariates as well as their interaction, *β*-coefficients, *SE* and 95% confidence intervals were rounded to three decimal places instead of two decimal places to improve interpretability.

[d] Standard error or lower bound of confidence interval equals zero due to rounding.

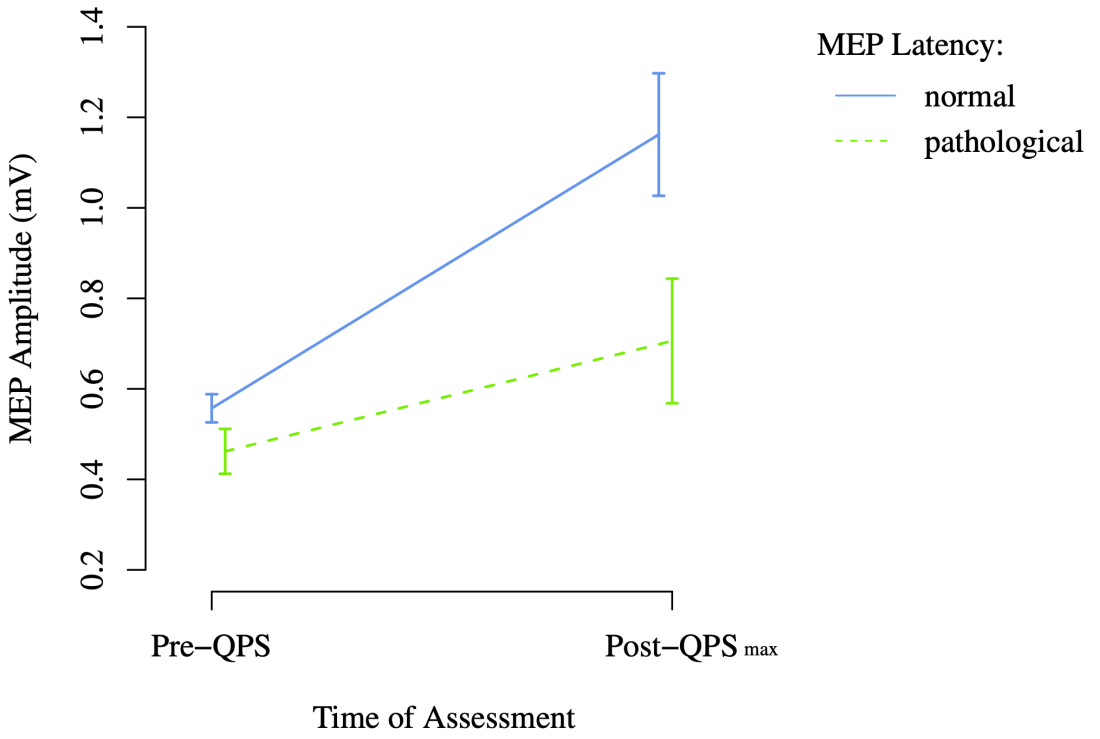

Fig. 6: MEP Amplitude from Pre-QPS to Post-QPS$_{max}$ Assessment by MEP Latency Duration.
The figure illustrates the QPS-induced increment in mean MEP amplitude in mV from pre- to post-QPS$_{max}$ assessment of participants with normal (< 24.5 ms) and pathological (> 24.5 ms) MEP latencies. The line representing the pathological latency group was slightly shifted to the right so that error bars and can better be identified. Abbreviation: max, maximum; ms, milliseconds; mV, millivolts. *Note*. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 08. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

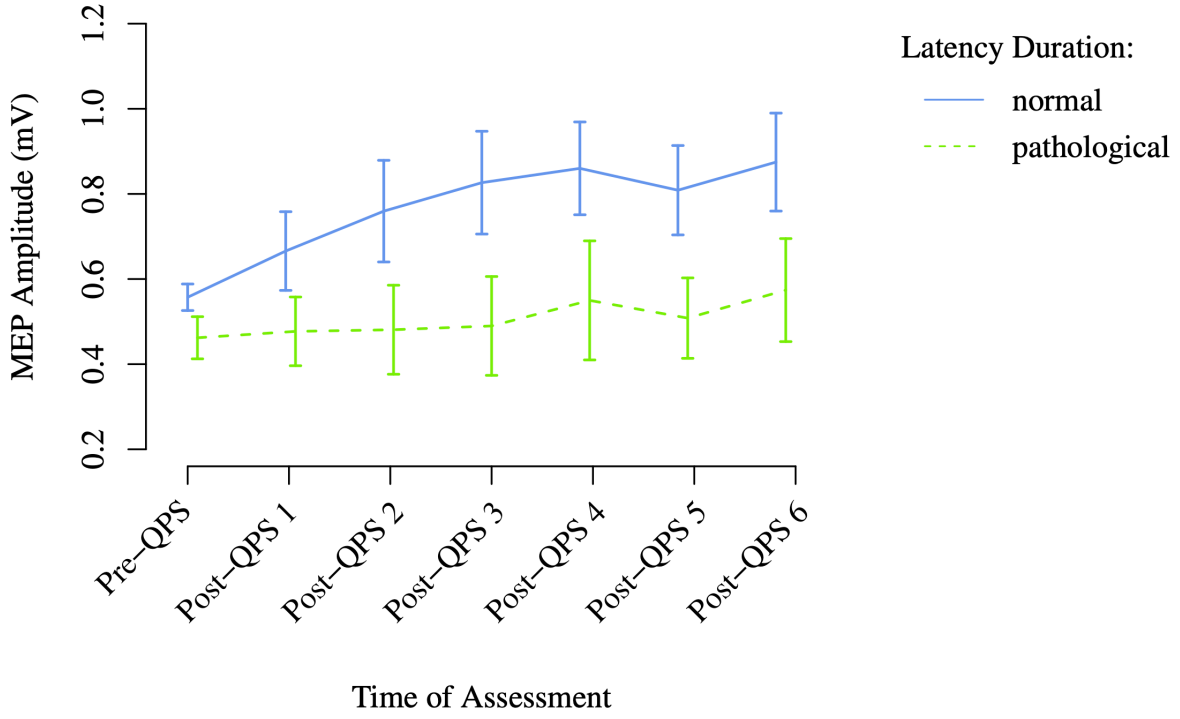

Fig. 7: MEP Amplitude across all Time points of Assessment by MEP Latency Duration.
The figure illustrates the QPS-induced increment in mean MEP amplitude in mV from pre QPS assessment across all post-QPS assessments (post-$QPS_{1\text{-}6}$) of participants with normal (< 24.5 ms) and pathological (> 24.5 ms) MEP latencies. The line representing the pathological latency group was slightly shifted to the right so that error bars can better be identified. Abbreviation: ms, milliseconds; mV, millivolts. *Note*. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 08. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

3.2.4 Exploratory Analysis

As for the main analysis, observed and predicted means of the explorative model are shown in **Table 7** and parameter information of the fixed and random effects are illustrated in **Table 8**. Furthermore, mean MEP amplitude of the four groups (PPMS, SPMS, RRMS and HC) at pre- and post-QPS_{max} assessment are illustrated in **Figure 8**. Likewise, mean MEP amplitudes of the four groups at pre- and post-$QPS_{1\text{-}6}$ assessments are illustrated in **Figure 9**. For clarity, when results of the exploratory analysis are matching those of the main analyses, only ANOVA results are shortly presented in the paragraphs below. However, deviating results as well as the main effect of group and the group by time interaction are reported in detail.

The fixed and random effects combined explained the same amount of data as the final model of the main analysis, marginal R^2 = .32, conditional R^2 = .97. Moreover, in accordance with the main analysis, there was a significant non-zero intercept $F(1,85) = 449.75$, $p < .001$. Likewise, there was a significant main effect of the time of assessment on mean MEP amplitude $F(1,85) = 32.57$, $p < .001$. In addition, regarding the random effects of time of assessment, mean MEP amplitude varied more strongly at post-QPS $Var_{post\text{-}QPSmax} = 0.25$, $SD = 0.50$, assessment compared to pre-QPS assessment, $Var_{pre\text{-}QPS} = 0.01$, $SD = 0.10$. This high variability of MEP amplitudes is again, also reflected by the increase in error bars from pre-QPS to post-QPS assessment in **Figure 8** and **Figure 9**.

Also, we observed no significant main effect of group on mean MEP amplitude $F(3,82) = 0.66$, $p = .577$. In that respect, the model revealed that neither PPMS, $b = -0.06$, $t(82) = -1.38$, $p = .172$, nor SPMS $b = -0.02$, $t(82) = -0.40$, $p = .689$, nor RRMS $b = -0.025$ $t(82) = -0.73$, $p = .465$, predict mean MEP amplitudes across time that are significantly different from those of HC. Moreover, in accordance with the main analysis, there was no significant interaction effect between time of assessment and group on mean MEP amplitude $F(3,85) = 0.54$, $p = .66$. Planned contrasts using Hochberg's correction revealed that the increment in MEP amplitude from pre- to post-QPS_{max} assessment did neither differ between participants with PPMS and SPMS $t(90) = 0.32$, $p = .961$, $d = 0.11$, nor between participants with PPMS and HC $t(90) = -1.09$, $p = .961$ $d = 0.58$, nor between participants with SPMS and HC $t(90) = -0.84$, $p = .961$, $d = 0.47$, nor between participants with PPMS and RRMS $t(90) = -1.05$, $p = .961$, $d = 0.58$, nor between participants with SPMS and RRMS $t(90) = -0.79$, $p = .961$, $d = 0.47$. In addition, the model showed that neither PPMS, $b = -0.16$, $t(85) = -1.01$, $p = .317$ nor SPMS $b = -0.08$, $t(85) = -0.53$, $p = .600$ nor RRMS $b = -0.02$, $t(85) = 0.18$, $p = .858$ did significantly predict increments in MEP amplitude from pre- to post-QPS_{max} assessment that are significantly different from those of HC. Again, the non-significance of these findings is reflected by the large overlapping error bars depicted in **Figure 8** and **Figure 9**.

Figure 8 shows a non-significant trend that might suggest that individuals with PPMS and SPMS have lower increments in post-QPS_{max} MEP amplitude than individuals with RRMS and HC. Yet, the figure shows that individuals with SPMS have slightly higher MEP amplitudes than individuals with PPMS. In addition, observed over the six time points, individuals with SPMS seem to show a somehow different dynamic in MEP amplitude increments compared to individuals with PPMS (**Figure 9**). Also, non-significant graphical trends depicted in **Figure 9** suggest that individuals with PPMS and SPMS might have higher increments in MEP amplitudes in the first half of the six time points of assessment compared to individuals with RRMS and HC. Still, for the majority of observations, MEP amplitudes of individuals with PPMS and SPMS are below those of HC and individuals with RRMS.

In accordance with main analysis, a significant main effect of the covariate mean MEP latency on mean MEP amplitude $F(1,82) = 6.04$, $p = .016$ was observed. Likewise, there was no significant main effect of age on MEP amplitude $F(1, 82) = 0.69$, $p = .410$. However, contrary to the main analysis, there was a significant interaction effect between mean MEP latency and age on mean MEP amplitude across times of assessment, $F(1, 82) = 4.34$, $p = .040$. This indicates, that across time points and groups, the magnitude of the influence of participants mean MEP latency on mean MEP amplitude did change depending on variations in participants' age. In that regard, the model showed that the magnitude of the influence of mean MEP latency on mean MEP amplitude significantly increased, when participants age increased above average, $b = 0.001$ $t(82) = 2.08$, $p = .040$.

Table 7: Observed and Predicted Means of the Explorative Multilevel Mixed Model.
Observed and explorative model predicted means of MEP amplitude by separated progressive groups.

MEP Amplitude (mV)	**Group**							
	PPMS (*n* = 14)		**SPMS (*n* = 20)**		**RRMS (*n* = 30)**		**HC (*n* = 30)**	
	Mean (*SE*)	**[95% CI]**	**Mean (*SE*)**	**[95% CI]**	**Mean (*SE*)**	**[95% CI]**	**Mean (*SE*)**	**[95% CI]**
Observed:								
Pre-QPS[a]	0.46 (0.03) {1.31}	-	0.51 (0.03) {1.42}	-	0.52 (0.02) {1.89}	-	0.57 (0.02) {1.89}	-
Post-QPS_{max}[a]	0.82 (0.15)	-	0.90 (0.12)	-	1.04 (0.10)	-	1.09 (0.09)	-
Predicted:								
Pre-QPS[a]	0.48 (0.04)	[0.41 ; 0.55]	0.52 (0.03)	[0.46 ; 0.59]	0.52 (0.02)	[0.47 ; 0.56]	0.54 (0.03)	[0.49 ; 0.59]
Post-QPS_{max}[a]	0.83 (0.14)	[0.56 ; 1.11]	0.96 (0.12)	[0.72 ; 1.20]	1.05 (0.10)	[0.86 ; 1.24]	1.05 (0.10)	[0.86 ; 1.25]

Note. Standard errors are between brackets. 95% confidence intervals are between squared brackets. Effect sizes (Cohen`s *d*) of within group differences are between curly brackets. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 06. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

[a] MEP amplitude at a stimulation intensity of the MEP 0,5 mV threshold.

Table 8: Parameter Information of the Explorative Multilevel Mixed Model.

Parameter information for fixed and random effects of the exploratory multilevel mixed model, comparing the maximum MEP amplitude after QPS with the MEP amplitude before QPS by separated progressive groups.

Parameter Information	**Fixed Effects**				**Random Effects**	
	***β*-coefficient (*SE*)**	**[95% CI]**	**Test Statistic**	***p***	**Variance**	***SD***
Intercept	**0.54** (0.03)	[0.49 ; 0.59]	$t(85) = 21.21$	**< .001**	-	-
Time of Assessment:						
Pre-QPS[a] [b]	-	-	-	-	-	-
Post-QPS_{max}[b]	**0.51** (0.09)	[0.33 ; 0.69]	$t(85) = 5.71$	**< .001**	-	-
Group:						
HC[a]	-	-	-	-	-	-
RRMS	- 0.03 (0.04)	[- 0.09 ; 0.04]	$t(82) = - 0.73$	.465	-	-
PPMS	- 0.06 (0.05)	[- 0.15 ; 0.03]	$t(82) = - 1.38$	.172	-	-
SPMS	- 0.02 (0.04)	[- 0.10 ; 0.07]	$t(82) = - 0.40$	.689	-	-
Covariates:						
Latency[c]	**- 0.011** (0.004)	[- 0.019 ; - 0.002]	$t(82) = - 2.47$	**.016**	-	-
Age[c]	0.001 (0.002)	[- 0.004 ; 0.002]	$t(82) = - 0.54$	.590	-	-
Interaction Terms:						
Post-QPS_{max} *RRMS	0.02 (0.13)	[- 0.23; 0.27]	$t(85) = 0.18$	.858	-	-
Post-QPS_{max} *PPMS	- 0.16 (0.16)	[- 0.47; 0.15]	$t(85) = - 1.01$	.317	-	-
Post-QPS_{max} *SPMS	- 0.08 (0.14)	[- 0.36; 0.21]	$t(85) = - 0.53$	.600		
Latency*Age[c] [d]	**0.001** (0.001)	[0.000; 0.002]	$t(82) = 2.08$	**.041**	-	-
Subject*Pre-QPS	-	-	-	-	0.01	0.10
Subject*Post-QPS_{max}	-	-	-	-	0.26	0.51
Residual	-	-	-	-	0.01	0.08

Note. Standard errors are between brackets. 95% confidence intervals of *β*-coefficients are between squared brackets and were estimated using bootstrapping method. Significant *β*-coefficients and *p*-values are highlighted in bold font. Conditional $R^2 = .97$. Marginal $R^2 = .32$. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 07. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

[a] Factor-level served as a reference group.

[b] MEP amplitude at a stimulation intensity of the MEP 0,5 mV threshold.

[c] Due to the small size of the estimated effect of the covariates as well as their interaction, *β*-coefficients, *SE* and 95% confidence intervals were rounded to three decimal places instead of two decimal places to improve interpretability.

[d] Lower bound of confidence interval equals zero due to rounding down to three decimal places.

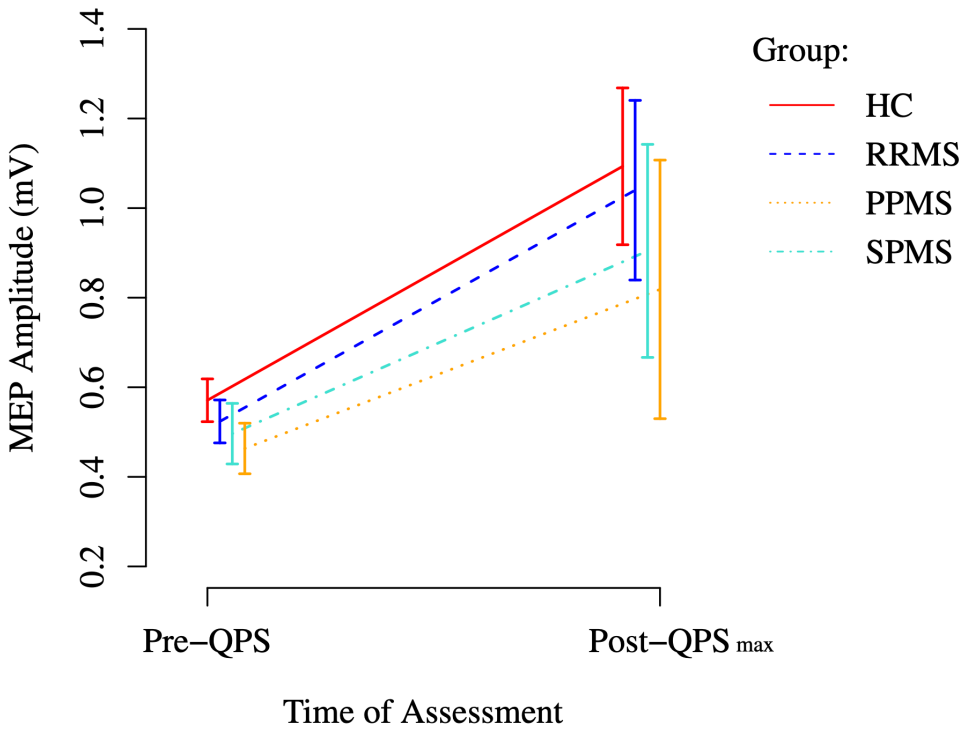

Fig. 8: MEP Amplitude from Pre-QPS to Post-QPS$_{max}$ Assessment by Group with Separated Subgroups. The figure illustrates the QPS-induced increment in mean MEP amplitude measured in mV from pre- to post-QPS$_{max}$ assessment of HC (n = 30), as well as participants with RRMS (n = 30), PPMS (n = 14) and SPMS (n = 20). The lines of the graph representing the RRMS, PPMS and SPMS group were slightly shifted to the right so that error bars and their overlap can better be identified. Abbreviation: max, maximum; mV, millivolts. *Note*. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 08. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

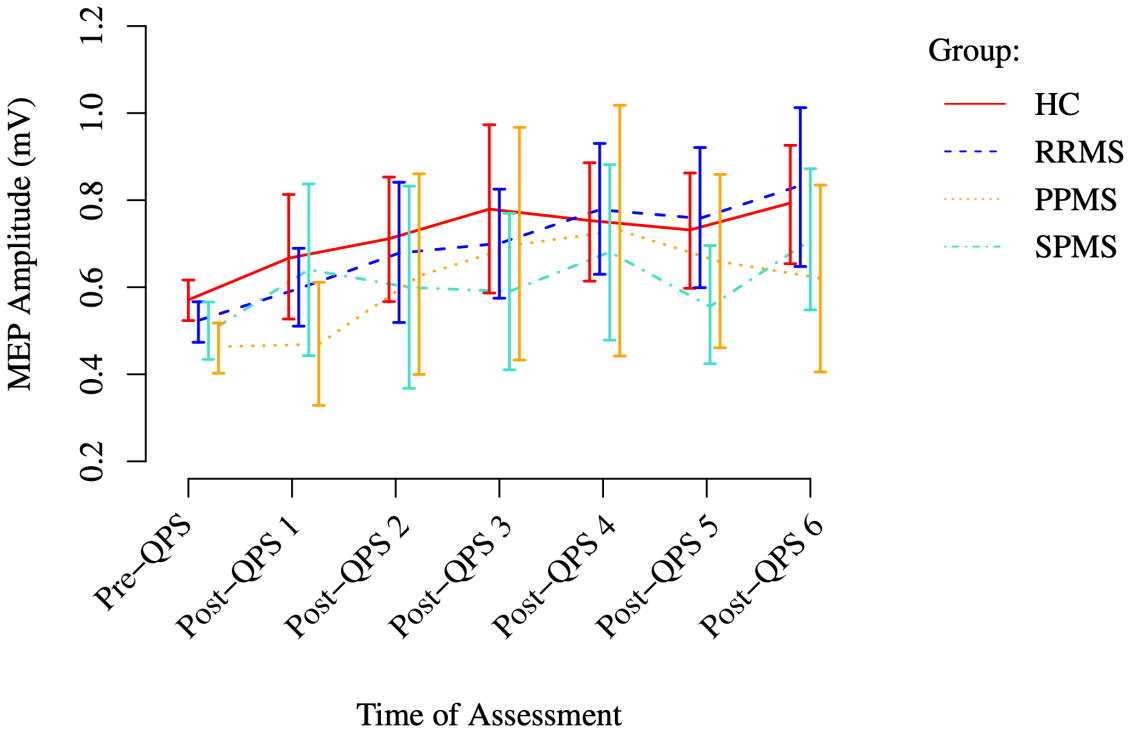

Fig. 9: MEP Amplitude across all Time points of Assessment by Group with Separated Subgroups.
The figure illustrates the QPS-induced increment in mean MEP amplitude measured in mV from pre QPS assessment across all post-QPS assessments (post-QPS$_{1\text{-}6}$) of HC (n = 30), as well as participants with RRMS (n = 30), PPMS (n = 14) and SPMS (n = 20). The lines of the graph representing the RRMS, PPMS, and SPMS group were slightly shifted so that error bars and their overlap can better be identified. Abbreviation: mV, millivolts. *Note*. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 08. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

3.2.5 Post Hoc Testing of the Exploratory Analysis

Due to the significant main effect of mean MEP latency on mean MEP amplitude, a post-hoc analysis was conducted, which revealed a significant interaction effect between time of assessment and mean MEP latency on mean MEP amplitude $F(1, 84) = 18.84$, $p < .001$. The statistical significance of all other fixed effects resembled the previous analysis. Overall, the refitted exploratory model represented the data better $AIC = 4.0$ than the original exploratory model $AIC = 20.0$ and the fixed effects explained more variance of the data, marginal $R^2 = .45$. For the interested reader, fixed and random effects of the model are depicted in **Table 9**.

Table 9: Parameter Information of the Post Hoc Exploratory Multilevel Mixed Model

Parameter information for fixed and random effects of the exploratory multilevel mixed model, comparing the maximum MEP amplitude after QPS with the MEP amplitude before QPS by separated progressive group, including the time by latency interaction.

Parameter Information	**Fixed Effects**				**Random Effects**	
	***β*-coefficient (*SE*)**	**[95% CI]**	**Test Statistic**	***p***	**Variance**	***SD***
Intercept	**0.54** (0.03)	[0.49 ; 0.59]	$t(84) = 21.03$	**< .001**	-	-
Time of Assessment:						
Pre-QPS[a b]	-	-	-	-	-	-
Post-QPS_{max}[c]	**0.41** (0.09)	[0.24 ; 0.58]	$t(84) = 4.82$	**< .001**	-	-
Group:						
HC[a]	-	-	-	-	-	-
RRMS	- 0.02 (0.03)	[- 0.09 ; 0.05]	$t(82) = - 0.62$	.536	-	-
PPMS	- 0.05 (0.05)	[- 0.14 ; 0.04]	$t(82) = - 1.06$	.291	-	-
SPMS	- 0.01 (0.04)	[- 0.09 ; 0.08]	$t(82) = - 0.18$	.858	-	-
Covariates:						
Latency[c]	**- 0.014** (0.004)	[- 0.022 ; - 0.005]	$t(82) = - 3.15$	**.002**	-	-
Age[c]	-0.001 (0.002)	[- 0.004 ; 0.002]	$t(82) = - 0.54$	.590	-	-
Interaction Terms:						
Post-QPS_{max} *RRMS	0.10 (0.12)	[- 0.13; 0.33]	$t(84) = 0.84$	.405	-	-
Post-QPS_{max} *PPMS	0.11 (0.15)	[- 0.19; 0.42]	$t(84) = 0.71$	.461	-	-
Post-QPS_{max} *SPMS	0.10 (0.14)	[- 0.17; 0.37]	$t(84) = 0.74$	.459		
Latency*Age[c d]	**0.001** (0.001)	[0.000; 0.002]	$t(82) = 2.08$	**.040**	-	-
Post-QPS*Latency	**- 0.06** (0.01)	[- 0.09; - 0.03]	$t(84) = - 4.34$	**< .001**		
Subject*Pre-QPS	-	-	-	-	0.01	0.10
Subject*Post-QPS_{max}	-	-	-	-	0.22	0.46
Residual	-	-	-	-	0.01	0.08

Note. Standard errors are between brackets. 95% confidence intervals of *β*-coefficients are between squared brackets and were estimated using bootstrapping method. Significant *β*-coefficients and *p*-values are highlighted in bold font. Conditional $R^2 = .97$. Marginal $R^2 = .32$. Adapted from "The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis" by C. Balloff, P. Albrecht, A.-S. Stucke, L. Scala, S. Novello, C.J. Hartmann, S.G. Meuth, A. Schnitzler, I.-K. Penner and S.J. Groiss, 2023, *Frontiers in Neurology*, 14, p. 08. (https://doi.org/10.3389/fneur.2023.1266225). Copyright 2023 by Balloff, Albrecht, Stucke, Scala, Novello, Hartmann, Meuth, Schnitzler, Penner and Groiss.

[a] Factor-level served as a reference group.

[b] MEP amplitude at a stimulation intensity of the MEP 0,5 mV threshold.

[c] Due to the small size of the estimated effect of the covariates as well as their interaction, *β*-coefficients, *SE* and 95% confidence intervals were rounded to four decimal places instead of three decimal places to improve interpretability.

[d] Lower bound of confidence interval equals zero due to rounding down to three decimal places.

4. Discussion and Conclusions

4.1 Summary

The aim of this thesis was to investigate, whether the degree of QPS-induced LTP-like plasticity differs between progressive subtypes of MS, relapsing remitting MS as well as healthy individuals and can therefore, serve as a suitable biomarker for MS. In that regard, a study was conducted in which it was hypothesized that QPS induces LTP-like plasticity in all of the above-mentioned groups (H_1), that individuals with stable RRMS do not differ in their degree of QPS-induced LTP-like plasticity from healthy individuals (H_2) and that individuals with PMS have lower degrees of QPS-induced LTP-like plasticity than individuals with stable RRMS (H_{3a}) and healthy individuals (H_{3b}). In line with H_1, this study demonstrated that QPS indeed induces LTP-like plasticity in all individuals regardless of group membership.

Moreover, in accordance with H_2, this study also demonstrated that individuals with stable RRMS do not differ in their degree of QPS-induced LTP-like plasticity from healthy individuals. Yet, contrary to H_{3a} and H_{3b}, this study shows that individuals with PMS did not show significantly lower degrees in QPS-induced LTP-like plasticity than individuals with RRMS or healthy individuals. This finding remained the same when individuals with PPMS and SPMS were exploratively analyzed as separate PMS groups. However, there was a high variability in post-QPS scores, and graphical trends suggest that individuals with PMS may have lower LTP-like plasticity than either individual with RRMS or HC. These null-findings may be due to the small to medium sample size of subgroups (HC n = 30, RRMS n = 30 and PMS n = 34) and thus, lack of statistical power. Nevertheless, exploratory analyses indicated, that differences in pyramidal tract integrity determine differences in QPS induced LTP-like plasticity, with lower degrees of pyramidal tract integrity resulting in lower QPS induced LTP-like plasticity increments. In addition, exploratory analyses indicate that the influence of pyramidal tract integrity on QPS induced cortical plasticity increases, to a small extent, with increasing age. However, before conclusions can be drawn, the results found here need to be interpreted in the context of the findings of other studies in this field, as well as this study's strengths and limitations.

4.2. Discussion

4.2.1 Interpretation of Results in Light of Previous Research

First, the results of this study are interpreted in the context of other research in the field, using the same or different LTP-like plasticity inducing rTMS protocols. The finding that QPS induces LTP-like plasticity in all individuals regardless of MS subtype is in line with previously conducted research, which indicated that QPS is a powerful tool in inducing LTP-like plasticity (Tiksnadi et al., 2020, Hamada et al., 2008). In addition, the finding that individuals with stable RRMS do not differ in their degree of QPS induced LTP-like plasticity from healthy individuals, is consistent with other findings from our research group, in which the same QPS protocol was used (Balloff et al., 2022, Balloff et al., 2023b). This finding is also concurring with those of studies, in which LTP-like plasticity was induced by means of other rTMS protocols such as PAS (Zeller et al., 2010) and iTBS (Mori et al., 2013). Given that it has been shown by our and other research groups, that individuals with RRMS who are in relapse or otherwise cognitively or motorically impaired, exhibit decreased LTP-like plasticity (Mori et al., 2014a, Mori et al., 2012, Balloff et al., 2022, Balloff et al., 2023b, Balloff et al., 2024), the result of this study supports the notion, that individuals with stable MS show preserved LTP-like plasticity, because inflammatory processes that impede plasticity are not as pronounced (Stampanoni Bassi et al., 2019b). However, a more recent study by Stampanoni Bassi et al. (2024) demonstrated lower levels of iTBS-induced LTP-like plasticity in recently diagnosed individuals with stable RRMS compared to HC, challenging the abovementioned claim. Yet, it has to be noted, that unlike the individuals in this study's sample, none of the individuals in the sample received anti-inflammatory or disease modifying medication prior to investigation (Stampanoni Bassi et al., 2024). In that regard, it was demonstrated that anti-inflammatory as well as disease modifying medication reduce neuroinflammation in MS (Romme Christensen et al., 2019), that the discontinuation of these medication predicts disease worsening and progression in individuals with stable RRMS (Jakimovski et al., 2022) and that anti-inflammatory treatment restores LTP-like plasticity in individuals with RRMS with active lesions (Mori et al., 2012). Taking these findings into account, the result of reduced LTP-like

plasticity in individuals with stable RRMS found by Stampanoni Bassi et al. (2024), may have been carried by a latently active inflammatory reaction due to non-treatment. Still, another conflicting result was reported by Baione et al. (2020), who in comparison with healthy individuals, found lower degrees of iTBS induced LTP-like plasticity in a medicated stable RRMS sample. The authors attributed these findings on methodological differences in the rTMS application, as well as inflammatory reactions, although no inflammatory markers were investigated (Baione et al., 2020). Consequently, to disentangle the relationship between QPS-induced LTP-like plasticity and neuroinflammation, future studies that use QPS and also consider inflammatory markers derived from MRI images and/or liquid markers from blood and CSF samples are warranted (Nazeri et al., 2022).

In addition, contrary to the finding of Mori et al. (2013) who induced LTP-like plasticity using iTBS, this study did neither find a difference in QPS-induced LTP-like plasticity between individuals with PMS and individuals with RRMS nor between individuals with PMS and HC. This is also striking, as the protocol used in this study monitored LTP-like plasticity in accordance with Nakamura et al. (2016), over the course 60 minutes. Therefore, the probability of capturing LTP-like plasticity, should have been higher compared to other protocols with shorter observation periods, such as Mori et al. (2013), who only monitored LTP-like plasticity over 15 minutes. Moreover, these results remained the same when the PPMS and SPMS groups were analyzed separately, ruling out the possibility that the null result was caused by the merger of these subgroups. This finding is striking, as previous studies using other LTP-inducing rTMS protocols found that individuals with PMS have low concentrations of platelet-derived growth factor (PDGF) and high concentrations of TNF-α in their CSF (Rossi et al., 2014, Mori et al., 2013), which are associated with neurodegeneration and decreased LTP-like plasticity (Rossi et al., 2014, Mori et al., 2014b). Hence, there might be other reasons for the null-finding in this study. One reason could have been the high variability in MEP amplitudes at post-QPS_{max} assessment and, as a consequence, loss in statistical power (Tu et al., 2005, Mullineaux and Wheat, 2017). This assumption is supported by the fact that non-significant graphical trends were observed, suggesting that individuals with PMS may have a lower average post-QPS_{max}, as well as post-QPS_{1-6} MEP amplitudes than both, healthy individuals and individuals with RRMS. In this study, the increased variability and statistical power loss, may have been caused by the comparably low number of 12 applied rTMS stimuli (± 9 after frame exclusion due to artifacts) (Goldsworthy et al., 2016, Chang et al., 2016, Biabani et al., 2018, Bashir et al., 2017), as well as the manual rTMS application (Richter et al., 2013, Kahl et al., 2023, Julkunen et al., 2009). To this respect, Chang et al. (2016) showed that depending on the rTMS protocol, 21 – 25 stimuli need to be applied in order to receive reliable measures of MEP amplitude. This is concurrent with other studies indicating that over 20 or even over 30 stimuli should be applied for the attainment of reliable MEP amplitudes (Goldsworthy et al., 2016, Biabani et al., 2018, Bashir et al., 2017). Only one meta-analysis by Cavaleri et al. (2017) suggests that the application of 10 stimuli might result in sufficiently reliable MEP amplitudes. This meta-analysis is however, solely based on four studies of which three focus on a different target muscle and two have a sample size of 12 subjects or less (Cavaleri et al., 2017).

Taken together, this difference in the number of applied stimuli could explain why Mori et al. (2013), who applied 25 rTMS stimuli, did find reduced LTP-like plasticity in PMS individuals, while Conte et al. (2009), who used 20 rTMS stimuli did not. In addition, regarding the manual application of rTMS, it was demonstrated that MRI based navigated rTMS results in a more precise identification of the motor hot spot (Caulfield et al., 2022), as well as the

stimulation of the target site over time (Richter et al., 2013) and thus, more reliable results of MEP amplitude (Kahl et al., 2023, Julkunen et al., 2009). Indeed, Bardel et al. (2024), who applied navigated rTMS, found that compared to individuals with RRMS, individuals with PPMS show reduced MEP amplitudes, suggesting that the trend found in this study may be due to variability caused by inconsistent coil positioning. Yet, the authors did not use a plasticity inducing rTMS protocol and thus, it remains unclear if this finding holds after plasticity inducing rTMS (Bardel et al., 2024). As a consequence, future studies using a navigated LTP-like plasticity inducing QPS protocol and a sufficiently high number of rTMS stimuli are needed to determine whether individuals with PMS have lower QPS-induced LTP-like plasticity than healthy individuals and individuals with stable RRMS, or not.

Moreover, exploratory analyses found that differences in pyramidal tract integrity as measured by means of MEP latency, determines differences in QPS-induced LTP-like plasticity. In this regard, analyses demonstrated that lower degrees of pyramidal tract integrity resulted in lower QPS-induced LTP-like plasticity increments. Moreover, exploratory analyses demonstrated that this influence of pyramidal tract integrity on QPS-induced LTP-like plasticity slightly grows with increasing age. These findings are in line with previous findings of our research group, in which a negative correlation between QPS-induced LTP-like plasticity and MEP latency was demonstrated (Balloff et al., 2022). Likewise, previous research from our and other research groups demonstrated that increasing age is associated with lower amounts of QPS-induced LTP-like plasticity (Balloff et al., 2022) and longer MEP latency (Shibuya et al., 2016). These associations, as well as the finding of this study could be explained by the concept of trans-synaptic degeneration (Murphy et al., 2023). This concept describes a process in which axonal or neuronal injury, for example due to inflammatory reactions, results in a loss of afferent and efferent neuronal connections, which in turn cause a degeneration of synaptically connected neurons (Murphy et al., 2023, Dinkin, 2017). In that regard, the axonal injury may result in a prolonged MEP latency (Jang et al., 2005), while the loss of synapses and neurons could express itself in a lower capacity to produce LTP-like plasticity (Wegner et al., 2006, Mori et al., 2013, Boonstra et al., 2020). In addition, as these processes accumulate over time, they could show an association with the affected individuals age (Pitcher et al., 2003, Musella et al., 2018, Manouchehrinia et al., 2017). Indeed, studies reported the loss of synapses and neurons in the spinal cord and cortex of individuals with PMS (Petrova et al., 2020, Jürgens et al., 2016). In accordance with that finding, this study revealed that MEP latencies were significantly more often pathological in individuals with PMS than healthy individuals and individuals with stable RRMS. Similarly, non-significant graphical trends of this study demonstrated lower degrees in LTP-like plasticity in individuals with PMS compared to healthy individuals and individuals with stable RRMS.

However, if trans-synaptic degeneration is indeed more frequent in individuals with PMS, the question arises as to why no statistically significant differences in MEP amplitudes were observed between these individuals and the other groups. An explanation could be that the individuals within the subgroups of MS do not form a homogenous group (Signori et al., 2023). This is supported by previous studies of our research group, showing that there are differences in QPS-induced LTP-like plasticity between cognitively and motorically high and low functioning individuals with RRMS (Balloff et al., 2022, Balloff et al., 2023b). Amongst other things, reasons for this heterogeneity could be differences in the response to pharmacological treatment and disease activity reflected by the amount of neuroinflammation and neurodegeneration (Signori et al., 2023, Mori et al., 2013, Luchetti et al., 2018, Cellerino

et al., 2021). Taking this into account, the abovementioned non-significant PMS subgroup trend may have resulted because the generally more commonly neurodegenerative processes in this MS subgroup are not expressed to the same degree in all individuals (Vavasour et al., 2022, Signori et al., 2023, Mahad et al., 2015). In any case, this heterogeneity may have added to the variability of post-QPS_{max} and post-QPS_{1-6} MEP amplitudes. Consequently, future studies are needed to focus more strongly on subgroup differences in pyramidal tract integrity by means of MEP latency and the impact of these differences on QPS-induced LTP like plasticity. Moreover, in order to reduce heterogeneity, future QPS studies should include markers of neuroinflammation and neurodegeneration, as well as sufficiently powered subgroup analyses of cognitively and motorically high and low functioning individuals.

4.2.2 Strengths and Limitations

Starting with the strengths, the study reported here is the first that assessed whether QPS-induced LTP-like plasticity differs between individuals with RRMS, PMS, and healthy individuals and can therefore, be considered as a diagnostic und prognostic biomarker of MS, which is not conflicted by the CRP. Although no difference in the amount of LTP-like plasticity between the three groups was found, this study is the first to reveal that the degree of pyramidal tract integrity determines QPS induced LTP-like plasticity and that this influence increases with increasing age.

Yet, the power of QPS compared to other rTMS protocols, lies in its higher reliability and lower non-responder rate of LTP-like plasticity induction (Tiksnadi et al., 2020). These advantages of QPS are assumed to result from the more selective activation of glutaminergic synapses within the M1 (Matsumoto and Ugawa, 2020), which appear to be distorted in MS (Macrez et al., 2016, Azevedo et al., 2014). Accordingly, these strengths of QPS compared to other rTMS protocols underscore the relevance of the graphical trends observed in this study. To this respect, it supports the notion that the high variability of LTP-like plasticity observed here, can rather be attributed to other factors, such as the low number of averaged stimuli and the non-navigated application (Richter et al., 2013, Kahl et al., 2023, Julkunen et al., 2009, Chang et al., 2016). Consequently, using QPS protocols seems to be the right endeavor to further investigate LTP-like plasticity in MS individuals.

Another strength lies in the conceptualization of this study. In that regard, the number of individuals with progressive MS included in this study exceeded those of previous studies in the field, which disregarding the low number of stimuli, added statistical power to the study design (Mori et al., 2013, Conte et al., 2009). Moreover, in addition to LTP-like plasticity, this study also focused on individuals' cognitive and motor functioning as well as their neurophysiological and psychiatric characteristics. The inclusion of these characteristics as covariates to the multilevel mixed model and the evaluation of their impact by means of backward elimination may have reduced confounding and thus, increased the internal-validity of this study (Van der Weele, 2019, Grimes and Schulz, 2002). Additionally, as LTP-like plasticity was monitored over the course of 60 minutes, the probability of capturing the maximum LTP-like plasticity, which had been shown to vary inter-individually was maximized, adding validity and power to the design (Ziemann and Siebner, 2015, Nakamura et al., 2016).

Yet, another strength of this study lies in the methodological approach. Namely, the comparison of the amount of LTP-like plasticity of PMS individuals with the amount of LTP-like plasticity of two control groups: healthy individuals and individuals with stable RRMS.

The inclusion of these two control groups benefits the internal validity of the study and supports the assumption that the observed trend of reduced QPS-induced LTP-like plasticity is caused by increased disease severity and distinct pathological processes in PMS individuals (Malay and Chung, 2012). Yet, it has to be mentioned that the benefit of these two control groups mostly accounts for the ANOVA results and less for the regression part of the conducted multilevel mixed model. However, as the observed increments in LTP-like plasticity were equal in individuals with RRMS and healthy individuals, an additional regression analysis in which the RRMS group was coded as a refence group did not seem feasible. Despite all of the abovementioned strengths of the reported study, there are also limitations that will be discussed next.

As mentioned above one limitation of this study is the relatively high interindividual variability of post-QPS_{max} MEP amplitudes in each group. This variability is surprising, as previous research demonstrated that QPS induces MEP amplitudes with only little fluctuation in comparison to other rTMS protocols (Tiksnadi et al., 2020, Nakamura et al., 2016). However, this lower variability has so far only been demonstrated in healthy individuals by the same research group in Fukushima (Tiksnadi et al., 2020, Nakamura et al., 2016, Matsumoto and Ugawa, 2020). Thus, further research needs to address whether this advantage of QPS generalizes or only accounts for certain forms of administration, ethnicities or population subgroups (Pellegrini et al., 2018). In any case, the variability of post-QPS_{max} MEP amplitudes in this study may possibly have overshadowed a significant between group effect of QPS-induced LTP-like plasticity and thus, needs further attention. As described above, the variability observed here may have been caused by the low number of 12 averaged stimuli at each trial (Chang et al., 2016). Yet, it has to be noted that this cohort study was part of a longitudinal study, which consisted of an extensive research design that took several hours to be conducted. Consequently, if more stimuli had been added to this study, it would have increased conductance time even further and thus, could have itself had detrimental effects on MEP amplitude variability due to increased fatigue/decreased alertness (Noreika et al., 2020, Guerra et al., 2020b).

Another reason mentioned above that may have resulted in increased variability of post-QPS_{max} MEP amplitudes could have been the incorrect identification of the motor hotspot or inconsistent stimulation of the target site due to the manual application of rTMS (Richter et al., 2013, Kahl et al., 2023, Julkunen et al., 2009). Although the correct manual stimulus application was persistently monitored by the trained investigators and the motor hotspot was marked with a colorful pen before the QPS protocol was started, navigated rTMS allows for a more precise identification and more constant stimulation of the target site (Julkunen et al., 2009). However, MRI images that are required for this navigated approach were not available in this study (Ruohonen and Karhu, 2010). Furthermore, adding the acquisition of these images to the study design would have prolonged the extensive protocol used here and thus, may have deterred participants from participation or biased the rTMS results due to increased fatigue/decreased alertness (Noreika et al., 2020, Guerra et al., 2020b).

An additional factor that may have contributed to the increased variability in MEP amplitudes is that this study applied an open-loop QPS protocol (Janssens and Sack, 2021). According to Leite et al. (2017), open-loop protocols are defined as rTMS techniques in which a stimulus of a predefined fixed intensity and interval is applied without considering the activity state of the targeted brain structure. This activity state of the brain is reflected by endogenous neural oscillations, that can be made visual in electroencephalography (EEG) (Zrenner et al.,

2018, Soleimani et al., 2023, Humaidan et al., 2024). In contrast, closed-loop rTMS protocols are those that apply rTMS in a brain-state dependent manner (Zrenner and Ziemann, 2024, Humaidan et al., 2024). That means that for every individual, each TMS stimulus is adapted according to the EEG-TMS output of the previous stimulus in an offline or real-time machine-learning-based approach (Humaidan et al., 2024). Consequently, stimuli are only applied when the stimulated brain structure is most susceptible for it (Zrenner et al., 2018). Due to this, closed loop rTMS protocols are highly personalized and thus, allow for a more precise and efficient application of stimuli (Zrenner et al., 2018, Tervo et al., 2022, Humaidan et al., 2024). This increased precision and efficiency is reflected in reduced variability of the measured TMS outcomes (Zrenner et al., 2018, Meincke et al., 2016). Nevertheless, a great body of closed-loop rTMS research has only emerged in the past few years before or even after this study was conceptualized and the development of valid closed-loop rTMS protocols is still in process (Humaidan et al., 2024, Hernandez-Pavon et al., 2023b, Carè et al., 2024). Also, the aim of this study was to use an established QPS protocol and apply it to a sample of individuals with different MS subtypes. It is therefore the task of future studies to develop a closed-loop QPS protocol and evaluate its effects in MS subgroups.

In addition, previous research demonstrated many other traits that influence MEP amplitude such as age (Pitcher et al., 2003), attention during rTMS application (Noreika et al., 2020), MEP latency (Vallence et al., 2023), biological sex (Pitcher et al., 2003), genetic make-up (Raginis-Zborowska et al., 2019), muscle contraction (Muellbacher et al., 2000), psychological disorders (Cantone et al., 2017), pharmacological treatment (Sohn et al., 2024), sleep (Lang et al., 2011), daytime of TMS application (Sale et al., 2007), as well as caffeine and nicotine intake amongst other things (Vigne et al., 2023, de Miquel et al., 2021). Many of these factors were controlled for in this study, either through covariate diagnostics (for example age, sex, MEP latency, pharmacological treatment, depressive symptoms and anxiety symptoms) or through the inclusion and exclusion criteria (for example, psychological disorders, pharmacological treatment and drug intake) while others were not considered (for example, genetic make-up, daytime of TMS application, sleep, caffeine and nicotine use) or not systematically assessed (for example attention during rTMS application and muscle contraction) (Van der Weele, 2019). However, it has to be noted that one study cannot control for all possible confounders, when type one error inflation ought to be prevented and a timely tolerable research design ought to be maintained (Wang et al., 2017, Van der Weele, 2019). In fact, this study pioneered in assessing and comparing QPS-induced LTP-like plasticity among individuals with different subtypes of MS and healthy individuals. Hence, it is the task of future studies to further evaluate factors that might impact the effects found here.

In addition, the merger of PPMS and SPMS could have contributed to the high variability in MEP amplitudes at post-QPS_{max} assessment. This is supported by the observed graphical trends, which demonstrated a different dynamic in MEP amplitude increments over time between individuals with PPMS and SPMS. However, the conducted exploratory analysis of this study did not show a significant difference in maximum LTP-like plasticity between both groups. In support of this, the observed graphical trends demonstrated only little deviations in the increment of MEP amplitude from pre-QPS to post-QPS_{max} between individuals with PPMS and SPMS. Besides, previous research indicates that while the clinical presentation differs (Lublin et al., 2014, Harding-Forrester et al., 2023), the pathophysiology of these two disease types shows a similar pattern (Lublin et al., 2014). Nevertheless, although the number of PPMS and SPMS participants was comparable to those of other studies (Mori et al., 2013,

Conte et al., 2009), it was probably too small to show a well powered between group effect if it was indeed there (Button et al., 2013). Thus, future studies should replicate these findings, using an adequate sample size to increase statistical power.

Further limitations concern the fact, that only the M1 was targeted with QPS in this study. However, in individuals with MS, this area can be affected by neurodegenerative processes (Bergsland et al., 2015). In that regard, studies indicated that surrounding areas such as the supplementary motor cortex often compensate for the loss in M1 functioning (Filippi et al., 2004, Faivre et al., 2015). As this compensatory reorganization of the brain poses a plastic change in itself (Prosperini et al., 2015, Pantano et al., 2006) and differs between MS subgroups (Loitfelder et al., 2011), it could also affect the ability to produce LTP-like plasticity (Stampanoni Bassi et al., 2019a). However, this structural and functional reorganization can only be assessed by imaging techniques such as functional MRI (Péran et al., 2020), which were not available in this study. Therefore, the impact of functional reorganization on LTP-like plasticity in different MS subgroups should be evaluated in future QPS studies.

Moreover, the QPS protocol of this study was only applied unilaterally to the left M1 and thus, only assessed LTP-like plasticity of the cortical networks in this region. Nevertheless, the inflammatory and neurodegenerative affection of the entire CNS may also impact intercortical networks connecting both hemispheres (Wahl et al., 2011, Tahedl et al., 2018). Indeed, studies demonstrate that intercortical networks play an important role in brain plasticity (Tamura et al., 2019, Bai et al., 2023). These intercortical networks can be assessed by adding fMRI to unilateral rTMS (Tamura et al., 2019), or by applying a bilateral rTMS protocol (Ferris et al., 2018). Therefore, applying these adaptations to future QPS studies may help to further reveal potential differences in LTP-like plasticity between different MS subgroups.

Another limitation is that only one specific type of plasticity, namely LTP-like plasticity was investigated in this study. Thus, based on the results found here, no inferences about other types of plasticity can be made. Nevertheless, assessing all types of plasticity was beyond the scope of this study. In addition, LTP-like plasticity is the most commonly investigated type of plasticity in MS research (Ksiazek-Winiarek et al., 2015). Reasons for this are that the affection of excitatory glutamate pathways through neuroinflammation are considered to be involved in MS pathology (Stampanoni Bassi et al., 2021, Levite, 2017) and that LTP-like plasticity was found to restore the function of neurons that were damaged as a consequence (Stampanoni Bassi et al., 2018, Mori et al., 2014a). Yet, an increasing body of research indicates that other types of plasticity, such as LTD and metaplasticity, may be of equal importance in neurodegenerative disorders (Zorumski and Izumi, 2012, Li et al., 2017, Kishore et al., 2012). For example, LTD-like plasticity was shown to be reduced in the cerebellum of mice with experimental autoimmune encephalomyelitis (Prochnow et al., 2013). In addition, Mori et al. (2013) demonstrated that while cTBS resulted in LTD-like plasticity in healthy individuals, it resulted in LTP-like plasticity in individuals with stable RRMS. Taken together, these findings point towards the conclusion that plasticity may not only be impaired in MS, but also dysregulated. Such a dysregulation might result from distortions in metaplasticity, a concept of activity dependent LTP and LTD plasticity regulation (Müller-Dahlhaus and Ziemann, 2015).
Indeed, Baione et al. (2020) demonstrated that metaplasticity was altered in an RRMS sample. Consequently, future QPS studies assessing LTP-like plasticity, LTD-like plasticity and metaplasticity are needed to disentangle the complex interplay of different plasticity types and their influence on MS pathology.

In addition, due to the brevity of the post-QPS assessment, which was conducted for a total period of 60 minutes, only early effects of LTP-plasticity were investigated (Raymond, 2007, Baltaci et al., 2019). While these early effects are assumed to also allow for inferences about more long-lasting, late effects (Zeller and Classen, 2014, Abraham et al., 1993), this might not be true in the presence neuroinflammation and neurodegeneration (Liu et al., 2012). Therefore, information about late effects is needed to uncover all facets of LTP-like plasticity in individuals with different subtypes of MS. In fact, when looking at the graphical trends of the six assessment points of this study, it seems as if LTP-like plasticity seems to increase beyond the investigated time frame. Thus, future studies should also focus on the long-lasting effects of QPS-induced LTP-like plasticity in different MS subtypes.

Furthermore, it needs to be stated that the HC group of the sample investigated here was a convenience sample, including friends as well as family members of the investigators and participants (Stratton, 2021). In addition, the study was conducted during the COVID-19 pandemic. This, together with the extensive test design, which may have also posed a barrier for severely affected MS individuals, could have resulted in the selection of fewer severely affected individuals with less weakened immune systems, participating in this study (Abraham et al., 2023). Indeed, the median EDSS in this study was moderate for individuals in the PMS group (Kurtzke, 1983). Moreover, in line with the findings by Sachdev et al. (2021), some of the individuals with MS who were approached to take part in this study stated as a reason for their unwillingness to participate, that they feared a COVID-19 infection when coming to the hospital for assessment. Also, as this study was conducted in an outpatient setting, some individuals, who were willing to participate could not do so, because they were too disabled to reach the clinic on their own. All of this may have contributed to a selection bias, causing systematic error and impeding generalizability of the results of this study (Tripepi et al., 2010, Cipriani et al., 2009). Nevertheless, it has to mentioned that some of this bias could be reduced by balancing participants in age, sex and education across all groups (Greifer and Stuart, 2021). Yet, the study should be replicated in a more severely affected MS sample to receive a complete picture regarding MS subgroup differences in QPS-induced LTP-like plasticity. However, due to the extensiveness of the protocol and the reduced baseline endurance of severely affected MS individuals, adherence to such a replication study may be low (Mead et al., 2012, Cavaleri et al., 2017). Thus, more patient friendly QPS protocols need to be developed to attract more disabled individuals to participate (Eggers et al., 2015).

Additionally, during this study, the TMS coils as well as the signal amplifier became defective and had to be sent to the manufacturer in order to be repaired. Due to this, recruitment and assessment had to be paused for about one year. However, as this study is cross-sectional and the pause was relatively short, no negative impacts on the results are assumed.

Moreover, it could be argued, that the sole comparison of the maximum post-QPS MEP amplitude may have caused systematic error due to the observed fluctuations of MEP amplitude. Having this in mind, it could further be argued that the grand mean of all six post-QPS assessment should have been compared instead. However quite the contrary is the case, as the time of LTP-like plasticity onset shows interindividual differences (Karabanov et al., 2015). The comparison of the grand mean of all six post-QPS assessments would thus have attenuated and overshadowed the full LTP-like plasticity inducing potential of QPS. Nonetheless, future studies evaluating between MS subgroup differences at all stages of QPS-induced LTP-like plasticity induction are needed. However, for that purpose a navigated, EEG guided, closed loop rTMS approach should be used in order to account for individual

differences in LTP-like plasticity occurrence and optimalization of LTP-like plasticity induction (Humaidan et al., 2024).

Despite of all limitations, it has to be noted that the study reported here was the first that examined LTP-like plasticity in different subtypes of MS, applying a QPS protocol. Taking the strengths and limitations of this study into account, it is the task of future studies to further investigate on these findings. Such avenues for future studies are outlined in the next chapter.

4.2. Outlook

The paragraphs before mentioned many ideas for adaptations through which future research could elaborate on the limitations of this study. For a better overview, this paragraph will provide a short summary of the most important implications. As stated above, these adaptations, should include an increased number of stimuli of minimally 25 for each trial to increase reliability (Chang et al., 2016). Moreover, inflammatory blood/CSF markers should be assessed to better evaluate the impact of disease activity in MS individuals on LTP-like plasticity (Nazeri et al., 2022). Furthermore, MRI should be included in future studies to allow for a more precise identification and stimulation of the targeted area through navigated rTMS (Julkunen et al., 2009, Caulfield et al., 2022). In addition, closed-loop protocols of QPS should be developed to account for interindividual variability in brain activity states and to increase efficiency of stimulus application (Humaidan et al., 2024). Also, bilateral rTMS should be applied to account for spatial variability in lesional load and inflammation as well as the impact of intercortical networks on LTP-like plasticity (Tamura et al., 2019, Ferris et al., 2018). Additionally, QPS protocols that assess other types of plasticity such as LTD-like plasticity and metaplasticity should be applied. Moreover, individuals with PPMS and SPMS should be investigated separately in a greater, higher-powered sample to assess whether there are indeed no differences in LTP-like plasticity between these subgroups. Furthermore, other factors, such as MEP latency (Vallence et al., 2023), attention (Noreika et al., 2020) and daytime of assessment (Sale et al., 2007), that impact LTP-like plasticity should more thoroughly be investigated and controlled for in the future. While this list could be continued, the most important aspects of future research are the focus on longitudinal studies, pharmacological studies and studies using personalized QPS approaches. Thus, the next paragraphs will briefly discuss these topics.

Up until now, no longitudinal study exists, that examined QPS-induced LTP like plasticity in individuals with different subtypes of MS over time. However, a subsequent study conducted by our research group investigated the relationship between LTP-plasticity at baseline and disease progression in a period of five years (Balloff et al., 2024). In that study, it was found that QPS-induced LTP-like plasticity at baseline did predict decline in some functional areas such as manual dexterity (Balloff et al., 2024). Yet, for other functional areas, either no effect was found or results remained inconclusive (Balloff et al., 2024). In any case, the number of stimuli used and the number of PMS individuals assessed in that study were at the lower end which may have impacted the power of the results (Goldsworthy et al., 2016, Chang et al., 2016, Biabani et al., 2018, Bashir et al., 2017, Balloff et al., 2024). Moreover, the median time span of assessment comprised only two years (Balloff et al., 2024). Yet, as MS progresses slowly, especially when treated adequately, longer time intervals are needed to assess the predictive value of QPS-induced LTP-like plasticity over time (Tremlett et al., 2006, Tedeholm et al., 2013). Nevertheless, longitudinal studies are important when assessing the

suitability of QPS-induced LTP-like plasticity as a prognostic biomarker of MS and should therefore be focused on in the future (Housley et al., 2015).

Another important aspect of future research is the impact of pharmacological and rehabilitative treatment on LTP-like plasticity in individuals with different subtypes of MS (Stampanoni Bassi et al., 2022). This is important because pharmacological and rehabilitative treatment positively effects neurotrophic and inflammatory processes (Sucksdorff et al., 2019, Mehrpour et al., 2015, Joisten et al., 2021). Indeed, these processes have been shown to affect the direction of rTMS induced LTP-like plasticity (Stampanoni Bassi et al., 2019b, Mori et al., 2013). Moreover, in line with these findings, studies indicate that rehabilitative treatment and some pharmacological treatment benefit LTP-like plasticity (Prosperini et al., 2015, Nicoletti et al., 2020). However, research on the latter is highly limited to non-MS medication (Stampanoni Bassi et al., 2018, Caipa et al., 2018). In addition, no other study systematically investigated the influence of these treatments on rTMS induced LTP-like plasticity in a well powered sample of individuals with different subtypes of MS. Still, research in this field is especially important to evaluate the ability of LTP-like plasticity as a prognostic biomarker of multiple sclerosis (Housley et al., 2015).

Furthermore, future studies should develop and implement personalized QPS protocols when assessing LTP-like plasticity in individuals with different subtypes of MS. According to Humaidan et al. (2024), these personalized protocols should consist of AI-based closed loop state dependent rTMS that is applied through multi locus TMS transducers. This recommendation is based on previous research indicating that the brain might be especially susceptible for LTP-plasticity induction during the negative peak of EEG measured alpha oscillations (Stefanou et al., 2018, Karabanov et al., 2021). These alpha oscillations have a frequency of about 8-13 Hz and also occur in the sensory motor cortex and the surrounding areas, where they are referred to as mu-rhythm (Llanos et al., 2013, Karabanov et al., 2021). Moreover, research indicates that multi locus TMS transducers improve feedback-controlled stimulation and allow for a faster detection of the target sites (Koponen et al., 2018). Thus, in summary, these personalized approaches not only increase reliability due to output optimalization (Menardi et al., 2022), but also may help to develop shorter, more patient friendly QPS protocols that are also accessible for more disabled individuals (Gogulski et al., 2023). Consequently, future research in this field is of utter importance when LTP-like plasticity should be established as a diagnostic and prognostic biomarker that is suitable in clinical practice.

4.3. Conclusions

This thesis evaluated, whether QPS-induced LTP-like plasticity can serve as a neurophysiological diagnostic and prognostic biomarker in different subtypes of MS. It can be concluded that QPS is a suitable and highly effective tool in inducing LTP-like plasticity not only in healthy individuals, but also in individuals with MS regardless of their subtype. Yet, based on the results, it cannot be concluded that the degree of QPS-induced LTP-like plasticity differs between individuals with different MS subtypes or between individuals with MS and healthy individuals. Rather, these results lead to the conclusion that the degree of QPS-induced LTP-like plasticity does more strongly depend on the degree of pyramidal tract integrity, which should further be investigated in future studies.

Nevertheless, non-significant graphical trends, showing that individuals with PMS have lower degrees of QPS-induced cortical plasticity than both healthy individuals and individuals

with stable RRMS, may point towards issues in statistical power of this study, for example due to methodological issues regarding the optimal rTMS application and number of applied TMS stimuli. In this regard, future longitudinal studies optimizing these methodological issues, such as through an AI-based personalized rTMS approach with a sufficiently high stimulus number, might help to better evaluate QPS-induced cortical plasticity as a MS biomarker and its potential in resolving the CRP. However, this thesis concludes that QPS-induced LTP-like plasticity cannot not yet be considered as a neurophysiological diagnostic and prognostic biomarker in different subtypes of MS.

References

.

ABDEL NASEER, M., RABAH, A. M., RASHED, L. A., HASSAN, A. & FOUAD, A. M. 2020. Glutamate and Nitric Oxide as biomarkers for disease activity in patients with multiple sclerosis. *Mult Scler Relat Disord,* 38**,** 101873.

ABRAHAM, S. A., AGYARE, D. F., YEBOA, N. K., OWUSU-SARPONG, A. A., BANULANZEKI, E. S., DOKU, D. T. & OBIRI-YEBOAH, D. 2023. The Influence of COVID-19 Pandemic on the Health Seeking Behaviors of Adults Living With Chronic Conditions: A View Through the Health Belief Model. *Journal of Primary Care and Community Health,* 14.

ABRAHAM, W. C., MASON, S. E., DEMMER, J., WILLIAMS, J. M., RICHARDSON, C. L., TATE, W. P., LAWLOR, P. A. & DRAGUNOW, M. 1993. Correlations between Immediate-Early Gene Induction and the Persistence of Long-Term Potentiation. *Neuroscience,* 56**,** 717-727.

AEBI, C. 2002. *Validierung Der neuropsychologischen Testbatterie CERAD-NP: eine multi-center studie.* University_of_Basel.

AGBOADA, D., MOSAYEBI-SAMANI, M., KUO, M. F. & NITSCHE, M. A. 2020. Induction of long-term potentiation-like plasticity in the primary motor cortex with repeated anodal transcranial direct current stimulation - Better effects with intensified protocols? *Brain Stimul,* 13**,** 987-997.

AKAIKE, H. 1987. Factor-Analysis and Aic. *Psychometrika,* 52**,** 317-332.

ALOISI, F., GIOVANNONI, G. & SALVETTI, M. 2023. Epstein-Barr virus as a cause of multiple sclerosis: opportunities for prevention and therapy. *Lancet Neurol,* 22**,** 338-349.

ARMSTRONG, R. A. 2014. When to use the Bonferroni correction. *Ophthalmic Physiol Opt,* 34**,** 502-8.

ARNETH, B. & KRAUS, J. 2022. Laboratory biomarkers of Multiple Sclerosis (MS). *Clin Biochem,* 99**,** 1-8.

ATTFIELD, K. E., JENSEN, L. T., KAUFMANN, M., FRIESE, M. A. & FUGGER, L. 2022. The immunology of multiple sclerosis. *Nat Rev Immunol,* 22**,** 734-750.

AZEVEDO, C. J., KORNAK, J., CHU, P., SAMPAT, M., OKUDA, D. T., CREE, B. A., NELSON, S. J., HAUSER, S. L. & PELLETIER, D. 2014. In Vivo Evidence of Glutamate Toxicity in Multiple Sclerosis. *Annals of Neurology,* 76**,** 269-278.

BAI, Z. F., ZHANG, J. J. & FONG, K. N. K. 2023. Intracortical and intercortical networks in patients after stroke: a concurrent TMS-EEG study. *Journal of Neuroengineering and Rehabilitation,* 20.

BAIONE, V., BELVISI, D., CORTESE, A., CETTA, I., TARTAGLIA, M., MILLEFIORINI, E., BERARDELLI, A. & CONTE, A. 2020. Cortical M1 plasticity and metaplasticity in patients with multiple sclerosis. *Mult Scler Relat Disord,* 38**,** 101494.

BALLOFF, C., ALBRECHT, P., STUCKE, A. S., SCALA, L., NOVELLO, S., HARTMANN, C. J., MEUTH, S. G., SCHNITZLER, A., PENNER, I. K. & GROISS, S. J. 2023a. The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis. *Front Neurol,* 14**,** 1266225.

BALLOFF, C., JANSSEN, L. K., HARTMANN, C. J., MEUTH, S. G., SCHNITZLER, A., PENNER, I. K. & ALBRECHT, P. 2024. Predictive value of synaptic plasticity for functional decline in patients with multiple sclerosis. *Front Neurol,* 15**,** 1410673.

BALLOFF, C., NOVELLO, S., STUCKE, A. S., JANSSEN, L. K., HEINEN, E., HARTMANN, C. J., MEUTH, S. G., SCHNITZLER, A., PENNER, I. K., ALBRECHT, P. & GROISS, S. J. 2023b. Long-term potentiation-like plasticity is retained during relapse in patients with Multiple Sclerosis. *Clin Neurophysiol,* 155**,** 76-85.

BALLOFF, C., PENNER, I. K., MA, M., GEORGIADES, I., SCALA, L., TROULLINAKIS, N., GRAF, J., KREMER, D., AKTAS, O., HARTUNG, H. P., MEUTH, S. G., SCHNITZLER, A., GROISS, S. J. & ALBRECHT, P. 2022. The degree of cortical plasticity correlates with cognitive performance in patients with Multiple Sclerosis. *Brain Stimul,* 15**,** 403-413.

BALTACI, S. B., MOGULKOC, R. & BALTACI, A. K. 2019. Molecular Mechanisms of Early and Late LTP. *Neurochemical Research,* 44**,** 281-296.

BARDEL, B., CREANGE, A., BONARDET, N., BAPST, B., ZEDET, M., WAHAB, A., AYACHE, S. S. & LEFAUCHEUR, J. P. 2024. Motor function in multiple sclerosis assessed by navigated transcranial magnetic stimulation mapping. *J Neurol,* 271**,** 4513-4528.

BARKER, A. T., JALINOUS, R. & FREESTON, I. L. 1985. Non-invasive magnetic stimulation of human motor cortex. *Lancet,* 1**,** 1106-7.

BARKHOF, F. 2002. The clinico-radiological paradox in multiple sclerosis revisited. *Curr Opin Neurol,* 15**,** 239-45.

BASHIR, S., YOO, W. K., KIM, H. S., LIM, H. S., ROTENBERG, A. & ABU JAMEA, A. 2017. The Number of Pulses Needed to Measure Corticospinal Excitability by Navigated Transcranial Magnetic Stimulation: Eyes Open vs. Close Condition. *Front Hum Neurosci,* 11**,** 121.

BECERRA, L., BISHOP, J., BARMETTLER, G., XIE, Y., NAVRATILOVA, E., PORRECA, F. & BORSOOK, D. 2016. Triptans disrupt brain networks and promote stress-induced CSD-like responses in cortical and subcortical areas. *J Neurophysiol,* 115**,** 208-17.

BENEDICT, R. H. B. 1997. *Brief visuospatial memory test - revised: professional manual.,* Odessa, FL, Psychological Assessment Resources, Inc.

BENEDICT, R. H. B., DELUCA, J., PHILLIPS, G., LAROCCA, N., HUDSON, L. D., RUDICK, R. & ASSESSM, M. S. O. 2017. Validity of the Symbol Digit Modalities Test as a cognition performance outcome measure for multiple sclerosis. *Multiple Sclerosis Journal,* 23**,** 721-733.

BENEDICT, R. H. B., DUQUIN, J. A., JURGENSEN, S., RUDICK, R. A., FEITCHER, J., MUNSCHAUER, F. E., PANZARA, M. A. & WEINSTOCK-GUTTMAN, B. 2008. Repeated assessment of neuropsychological deficits in multiple sclerosis using the Symbol Digit Modalities Test and the MS Neuropsychological Screening Questionnaire. *Multiple Sclerosis Journal,* 14**,** 940-946.

BENEDICT, R. H. B., SCHRETLEN, D., GRONINGER, L., DOBRASKI, M. & SHPRITZ, B. 1996. Revision of the brief visuospatial memory test: Studies of normal performance, reliability, and validity. *Psychological Assessment,* 8**,** 145-153.

BERGER, K., BROLL, S., WINKELMANN, J., HEBERLEIN, I., MULLER, T., RIES, V. & FAQT-STUDIENZENTREN 1999. Reliability analysis of the PDQ-39 (German version). *Aktuelle Neurologie,* 26**,** 180-184.

BERGSLAND, N., LAGANÀ, M. M., TAVAZZI, E., CAFFINI, M., TORTORELLA, P., BAGLIO, F., BASELLI, G. & ROVARIS, M. 2015. Corticospinal tract integrity is related to primary motor cortex thinning in relapsing-remitting multiple sclerosis. *Multiple Sclerosis Journal,* 21**,** 1771-1780.

BIABANI, M., FARRELL, M., ZOGHI, M., EGAN, G. & JABERZADEH, S. 2018. The minimal number of TMS trials required for the reliable assessment of corticospinal excitability, short interval intracortical inhibition, and intracortical facilitation. *Neurosci Lett,* 674**,** 94-100.

BJELLAND, I., DAHL, A. A., HAUG, T. T. & NECKELMANN, D. 2002. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. *J Psychosom Res,* 52**,** 69-77.

BLAKESLEY, R. E., MAZUMDAR, S., DEW, M. A., HOUCK, P. R., TANG, G., REYNOLDS, C. F., 3RD & BUTTERS, M. A. 2009. Comparisons of methods for multiple hypothesis testing in neuropsychological research. *Neuropsychology,* 23**,** 255-64.

BOESCHOTEN, R. E., BRAAMSE, A. M. J., BEEKMAN, A. T. F., CUIJPERS, P., VAN OPPEN, P., DEKKER, J. & UITDEHAAG, B. M. J. 2017. Prevalence of depression and anxiety in Multiple Sclerosis: A systematic review and meta-analysis. *J Neurol Sci,* 372**,** 331-341.

BOLOGNA, M., BERTRAM, K., PAPARELLA, G., PAPI, C., BELVISI, D., CONTE, A., SUPPA, A., WILLIAMS, D. R. & BERARDELLI, A. 2017. Reversal of long term potentiation-like plasticity in primary motor cortex in patients with progressive supranuclear palsy. *Clin Neurophysiol,* 128**,** 1547-1552.

BOONSTRA, F. M., NOFFS, G., PERERA, T., JOKUBAITIS, V. G., VOGEL, A. P., MOFFAT, B. A., BUTZKUEVEN, H., EVANS, A., VAN DER WALT, A. & KOLBE, S. C. 2020. Functional neuroplasticity in response to cerebello-thalamic injury underpins the clinical presentation of tremor in multiple sclerosis. *Mult Scler,* 26**,** 696-705.

BROOS, J. Y., VAN DER BURGT, R. T. M., KONINGS, J., RIJNSBURGER, M., WERZ, O., DE VRIES, H. E., GIERA, M. & KOOIJ, G. 2024. Arachidonic acid-derived lipid mediators in multiple sclerosis pathogenesis: fueling or dampening disease progression? *J Neuroinflammation,* 21**,** 21.

BROWNLEE, W. J. & MILLER, D. H. 2014. Clinically isolated syndromes and the relationship to multiple sclerosis. *J Clin Neurosci,* 21**,** 2065-71.

BURKI, C. N., LUDWIG, C., CHICHERIO, C. & DE RIBAUPIERRE, A. 2014. Individual differences in cognitive plasticity: an investigation of training curves in younger and older adults. *Psychol Res,* 78**,** 821-35.

BUTTON, K. S., IOANNIDIS, J. P. A., MOKRYSZ, C., NOSEK, B. A., FLINT, J., ROBINSON, E. S. J. & MUNAFÒ, M. R. 2013. Power failure: why small sample size undermines the reliability of neuroscience (vol 14, pg 365-376, 2013). *Nature Reviews Neuroscience,* 14**,** 444-444.

CACCHIO, A., PAOLONI, M., CIMINI, N., MANGONE, M., LIRIS, G., ALOISI, P., SANTILLI, V. & MARRELLI, A. 2011. Reliability of TMS-related measures of tibialis anterior muscle in patients with chronic stroke and healthy subjects. *J Neurol Sci,* 303**,** 90-4.

CAGNEY, D. N., SUL, J., HUANG, R. Y., LIGON, K. L., WEN, P. Y. & ALEXANDER, B. M. 2018. The FDA NIH Biomarkers, EndpointS, and other Tools (BEST) resource in neuro-oncology. *Neuro-Oncology,* 20**,** 1162-1172.

CAIPA, A., ALOMAR, M. & BASHIR, S. 2018. TMS as a tool to investigate the effect of pharmacological medications on cortical plasticity. *European Review for Medical and Pharmacological Sciences,* 22**,** 844-852.

CANTONE, M., BRAMANTI, A., LANZA, G., PENNISI, M., BRAMANTI, P., PENNISI, G. & BELLA, R. 2017. Cortical Plasticity in Depression: A Neurochemical Perspective From Transcranial Magnetic Stimulation. *Asn Neuro,* 9.

CARÈ, M., CHIAPPALONE, M. & COTA, V. R. 2024. Personalized strategies of neurostimulation: from static biomarkers to dynamic closed-loop assessment of neural function. *Frontiers in Neuroscience,* 18.

CAULFIELD, K. A., FLEISCHMANN, H. H., COX, C. E., WOLF, J. P., GEORGE, M. S. & MCTEAGUE, L. M. 2022. Neuronavigation maximizes accuracy and precision in TMS positioning: Evidence from 11,230 distance, angle, and electric field modeling measurements. *Brain Stimulation,* 15**,** 1192-1205.

CAVALERI, R., SCHABRUN, S. M. & CHIPCHASE, L. S. 2017. The number of stimuli required to reliably assess corticomotor excitability and primary motor cortical

representations using transcranial magnetic stimulation (TMS): a systematic review and meta-analysis. *Syst Rev,* 6**,** 48.
CELLERINO, M., BOFFA, G., LAPUCCI, C., TAZZA, F., SBRAGIA, E., MANCUSO, E., BRUSCHI, N., MINGUZZI, S., IVALDI, F., POIRE, I., LARONI, A., MANCARDI, G., CAPELLO, E., UCCELLI, A., NOVI, G. & INGLESE, M. 2021. Predictors of Ocrelizumab Effectiveness in Patients with Multiple Sclerosis. *Neurotherapeutics,* 18**,** 2579-2588.
CHANG, W. H., FRIED, P. J., SAXENA, S., JANNATI, A., GOMES-OSMAN, J., KIM, Y. H. & PASCUAL-LEONE, A. 2016. Optimal number of pulses as outcome measures of neuronavigated transcranial magnetic stimulation. *Clinical Neurophysiology,* 127**,** 2892-2897.
CHERIYAN, J., KIM, S., WOLANSKY, L. J., COOK, S. D. & CADAVID, D. 2012. Impact of inflammation on brain volume in multiple sclerosis. *Arch Neurol,* 69**,** 82-8.
CIPRIANI, A., PURGATO, M. & BARBUI, C. 2009. Why internal and external validity of experimental studies are relevant for clinical practice? *Epidemiologia E Psichiatria Sociale-an International Journal for Epidemiology and Psychiatric Sciences,* 18**,** 101-103.
CLAUS, D. 2007. Motorisch evozierte Potenziale der Bein- und Fußmuskeln. *In:* SIEBNER, H. R. & ZIEMANN, U. (eds.) *Das TMS-Buch: Handbuch der transkraniellen Magnetstimulation.* Berlin, Heidelberg: Springer Berlin Heidelberg.
COHEN, J. 1988. *Statistical power analysis for the behavioral sciences,* Hillsdale, NJ, Erlbaum.
COHEN, J. 1992. A power primer. *Psychological Bulletin,* 112**,** 155–159.
CONTE, A., LENZI, D., FRASCA, V., GILIO, F., GIACOMELLI, E., GABRIELE, M., BETTOLO, C. M., IACOVELLI, E., PANTANO, P., POZZILLI, C. & INGHILLERI, M. 2009. Intracortical excitability in patients with relapsing-remitting and secondary progressive multiple sclerosis. *J Neurol,* 256**,** 933-8.
CONTE, A., LI VOTI, P., PONTECORVO, S., QUARTUCCIO, M. E., BAIONE, V., ROCCHI, L., CORTESE, A., BOLOGNA, M., FRANCIA, A. & BERARDELLI, A. 2016. Attention-related changes in short-term cortical plasticity help to explain fatigue in multiple sclerosis. *Mult Scler,* 22**,** 1359-66.
CORP, D. T., BEREZNICKI, H. G. K., CLARK, G. M., YOUSSEF, G. J., FRIED, P. J., JANNATI, A., DAVIES, C. B., GOMES-OSMAN, J., STAMM, J., CHUNG, S. W., BOWE, S. J., ROGASCH, N. C., FITZGERALD, P. B., KOCH, G., DI LAZZARO, V., PASCUAL-LEONE, A., ENTICOTT, P. G. & BIG, T. M. S. D. C. 2020. Large-scale analysis of interindividual variability in theta-burst stimulation data: Results from the 'Big TMS Data Collaboration'. *Brain Stimul,* 13**,** 1476-1488.
CORREALE, J., GAITAN, M. I., YSRRAELIT, M. C. & FIOL, M. P. 2017. Progressive multiple sclerosis: from pathogenic mechanisms to treatment. *Brain,* 140**,** 527-546.
COUPE, P., PLANCHE, V., MANSENCAL, B., KAMROUI, R. A., KOUBIYR, I., MANJON, J. V. & TOURDIAS, T. 2023. Lifespan neurodegeneration of the human brain in multiple sclerosis. *Hum Brain Mapp,* 44**,** 5602-5611.
DE MIQUEL, C., PROSS, B., PAPAZOVA, I., GÜLER, D. & HASAN, A. 2021. The two-way relationship between nicotine and cortical activity: a systematic review of neurobiological and treatment aspects. *European Archives of Psychiatry and Clinical Neuroscience,* 271**,** 157-180.
DELVENDAHL, I., JUNG, N. H., KUHNKE, N. G., ZIEMANN, U. & MALL, V. 2012. Plasticity of motor threshold and motor-evoked potential amplitude--a model of intrinsic and synaptic plasticity in human motor cortex? *Brain Stimul,* 5**,** 586-93.
DENDROU, C. A., FUGGER, L. & FRIESE, M. A. 2015. Immunopathology of multiple sclerosis. *Nat Rev Immunol,* 15**,** 545-58.

DI IORIO, R. & ROSSINI, P. M. 2017. Safety Considerations of the Use of TMS. *In:* M. KRIEG, S. (ed.) *Navigated Transcranial Magnetic Stimulation in Neurosurgery.* Cham: Springer International Publishing.

DI LAZZARO, V., OLIVIERO, A., PROFICE, P., PENNISI, M. A., DI GIOVANNI, S., ZITO, G., TONALI, P. & ROTHWELL, J. C. 2000. Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. *Exp Brain Res,* 135**,** 455-61.

DIAMOND, D. M., DUNWIDDIE, T. V. & ROSE, G. M. 1988. Characteristics of hippocampal primed burst potentiation in vitro and in the awake rat. *J Neurosci,* 8**,** 4079-88.

DIAS DE SOUSA, M. A., DESIDERIO, C. S., DA SILVA CATARINO, J., TREVISAN, R. O., ALVES DA SILVA, D. A., ROCHA, V. F. R., BOVI, W. G., TIMOTEO, R. P., BONATTI, R. C. F., DA SILVA, A. E., FERNANDEZ, A. L., SALES-CAMPOS, H., RODRIGUES JUNIOR, V., DA SILVA, M. V. & DE OLIVEIRA, C. J. F. 2022. Role of Cytokines, Chemokines and IFN-gamma(+) IL-17(+) Double-Positive CD4(+) T Cells in Patients with Multiple Sclerosis. *Biomedicines,* 10.

DINKIN, M. 2017. Trans-synaptic Retrograde Degeneration in the Human Visual System: Slow, Silent, and Real. *Curr Neurol Neurosci Rep,* 17**,** 16.

DOBSON, R. & GIOVANNONI, G. 2019. Multiple sclerosis - a review. *Eur J Neurol,* 26**,** 27-40.

DOMINGUEZ-VILLAR, M., RADDASSI, K., DANIELSEN, A. C., GUARNACCIA, J. & HAFLER, D. A. 2019. Fingolimod modulates T cell phenotype and regulatory T cell plasticity in vivo. *J Autoimmun,* 96**,** 40-49.

DOSHI, A. & CHATAWAY, J. 2017. Multiple sclerosis, a treatable disease. *Clin Med (Lond),* 17**,** 530-536.

DUNN, O. J. 1961. Multiple Comparisons among Means. *Journal of the American Statistical Association,* 56**,** 52-&.

DUNSCHEDE, J., RUSCHIL, C., BENDER, B., MENGEL, A., LINDIG, T., ZIEMANN, U. & KOWARIK, M. C. 2023. Clinical-Radiological Mismatch in Multiple Sclerosis Patients during Acute Relapse: Discrepancy between Clinical Symptoms and Active, Topographically Fitting MRI Lesions. *J Clin Med,* 12.

EGGERS, C., GÜNTHER, M., ROTHWELL, J., TIMMERMANN, L. & RUGE, D. 2015. Theta burst stimulation over the supplementary motor area in Parkinson's disease. *Journal of Neurology,* 262**,** 357-364.

ELLIOTT, C., BELACHEW, S., WOLINSKY, J. S., HAUSER, S. L., KAPPOS, L., BARKHOF, F., BERNASCONI, C., FECKER, J., MODEL, F., WEI, W. & ARNOLD, D. L. 2019. Chronic white matter lesion activity predicts clinical progression in primary progressive multiple sclerosis. *Brain,* 142**,** 2787-2799.

ENGELHARDT, B., COMABELLA, M. & CHAN, A. 2022. Multiple sclerosis: Immunopathological heterogeneity and its implications. *Eur J Immunol,* 52**,** 869-881.

ESIRI, M. M. & CHANCE, S. A. 2012. Cognitive reserve, cortical plasticity and resistance to Alzheimer's disease. *Alzheimers Res Ther,* 4**,** 7.

FAIVRE, A., RICO, A., ZAARAOUI, W., REUTER, F., CONFORT-GOUNY, S., GUYE, M., PELLETIER, J., RANJEVA, J. P. & AUDOIN, B. 2015. Brain functional plasticity at rest and during action in multiple sclerosis patients. *Journal of Clinical Neuroscience,* 22**,** 1438-1443.

FDA-NIH BIOMARKER WORKING GROUP 2016. FDA-NIH Biomarker Working Group BEST (Biomarkers, EndpointS, and other Tools) Resource [Internet]. Silver Spring (MD): Food and Drug Administration (US); 2016-. Co-published by National Institutes of Health (US) Bethesda (MD).

FERRERI, F., PASQUALETTI, P., MAATTA, S., PONZO, D., FERRARELLI, F., TONONI, G., MERVAALA, E., MINIUSSI, C. & ROSSINI, P. M. 2011. Human

brain connectivity during single and paired pulse transcranial magnetic stimulation. *Neuroimage,* 54**,** 90-102.
FERRIS, J. K., NEVA, J. L., FRANCISCO, B. A. & BOYD, L. A. 2018. Bilateral Motor Cortex Plasticity in Individuals With Chronic Stroke, Induced by Paired Associative Stimulation. *Neurorehabilitation and Neural Repair,* 32**,** 671-681.
FILIPPI, M., ROCCA, M. A., MEZZAPESA, D. M., FALINI, A., COLOMBO, B., SCOTTI, G. & COMI, G. 2004. A functional MRI study of cortical activations associated with object manipulation in patients with MS. *Neuroimage,* 21**,** 1147-1154.
FILSER, M., SCHREIBER, H., POTTGEN, J., ULLRICH, S., LANG, M. & PENNER, I. K. 2018. The Brief International Cognitive Assessment in Multiple Sclerosis (BICAMS): results from the German validation study. *J Neurol,* 265**,** 2587-2593.
FIRMIN, L., MULLER, S. & ROSLER, K. M. 2012. The latency distribution of motor evoked potentials in patients with multiple sclerosis. *Clin Neurophysiol,* 123**,** 2414-21.
FISCHER, J. S., RUDICK, R. A., CUTTER, G. R. & REINGOLD, S. C. 1999. The Multiple Sclerosis Functional Composite Measure (MSFC): an integrated approach to MS clinical outcome assessment. National MS Society Clinical Outcomes Assessment Task Force. *Mult Scler,* 5**,** 244-50.
FITZGERALD, P. B., FOUNTAIN, S. & DASKALAKIS, Z. J. 2006. A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. *Clin Neurophysiol,* 117**,** 2584-96.
FLETCHER, J. M., LALOR, S. J., SWEENEY, C. M., TUBRIDY, N. & MILLS, K. H. 2010. T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. *Clin Exp Immunol,* 162**,** 1-11.
FORD, J. G., BREMNER, F. J. & RICHIE, W. R. 1970. The effect of hours of food deprivation on hippocampal theta rhythm. *Neuropsychologia,* 8**,** 65-73.
FRANCIOTTA, D., SALVETTI, M., LOLLI, F., SERAFINI, B. & ALOISI, F. 2008. B cells and multiple sclerosis. *Lancet Neurol,* 7**,** 852-8.
FRATELLO, F., VENIERO, D., CURCIO, G., FERRARA, M., MARZANO, C., MORONI, F., PELLICCIARI, M. C., BERTINI, M., ROSSINI, P. M. & DE GENNARO, L. 2006. Modulation of corticospinal excitability by paired associative stimulation: reproducibility of effects and intraindividual reliability. *Clin Neurophysiol,* 117**,** 2667-74.
FUJIWARA, T. & ROTHWELL, J. C. 2004. The after effects of motor cortex rTMS depend on the state of contraction when rTMS is applied. *Clin Neurophysiol,* 115**,** 1514-8.
GIFFROY, X., MAES, N., ALBERT, A., MAQUET, P., CRIELAARD, J. M. & DIVE, D. 2017. Do evoked potentials contribute to the functional follow-up and clinical prognosis of multiple sclerosis? *Acta Neurol Belg,* 117**,** 53-59.
GILIO, F., CONTE, A., VANACORE, N., FRASCA, V., INGHILLERI, M. & BERARDELLI, A. 2007. Excitatory and inhibitory after-effects after repetitive magnetic transcranial stimulation (rTMS) in normal subjects. *Exp Brain Res,* 176**,** 588-93.
GOGULSKI, J., ROSS, J. M., TALBOT, A., CLINE, C. C., DONATI, F. L., MUNOT, S., KIM, N., GIBBS, C., BASTIN, N., YANG, J., MINASI, C., SARKAR, M., TRUONG, J. & KELLER, C. J. 2023. Personalized Repetitive Transcranial Magnetic Stimulation for Depression. *Biological Psychiatry-Cognitive Neuroscience and Neuroimaging,* 8**,** 351-360.
GOLDMAN, M. D., MOTL, R. W., SCAGNELLI, J., PULA, J. H., SOSNOFF, J. J. & CADAVID, D. 2013. Clinically meaningful performance benchmarks in MS: timed 25-foot walk and the real world. *Neurology,* 81**,** 1856-63.
GOLDSWORTHY, M. R., HORDACRE, B. & RIDDING, M. C. 2016. Minimum number of trials required for within- and between-session reliability of TMS measures of corticospinal excitability. *Neuroscience,* 320**,** 205-9.

GOLDSWORTHY, M. R., MULLER-DAHLHAUS, F., RIDDING, M. C. & ZIEMANN, U. 2015. Resistant Against De-depression: LTD-Like Plasticity in the Human Motor Cortex Induced by Spaced cTBS. *Cereb Cortex,* 25**,** 1724-34.

GREIFER, N. & STUART, E. A. 2021. Matching Methods for Confounder Adjustment: An Addition to the Epidemiologist's Toolbox. *Epidemiologic Reviews,* 43**,** 118-129.

GRIMES, D. A. & SCHULZ, K. F. 2002. Bias and causal associations in observational research. *Lancet,* 359**,** 248-252.

GROPPA, S., OLIVIERO, A., EISEN, A., QUARTARONE, A., COHEN, L. G., MALL, V., KAELIN-LANG, A., MIMA, T., ROSSI, S., THICKBROOM, G. W., ROSSINI, P. M., ZIEMANN, U., VALLS-SOLE, J. & SIEBNER, H. R. 2012. A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. *Clinical Neurophysiology,* 123**,** 858-882.

GROSS, R. H., SILLAU, S. H., MILLER, A. E., FARRELL, C. & KRIEGER, S. C. 2019. The Multiple Sclerosis Severity Score: fluctuations and prognostic ability in a longitudinal cohort of patients with MS. *Mult Scler J Exp Transl Clin,* 5**,** 2055217319837254.

GUERRA, A., LOPEZ-ALONSO, V., CHEERAN, B. & SUPPA, A. 2020a. Variability in non-invasive brain stimulation studies: Reasons and results. *Neurosci Lett,* 719**,** 133330.

GUERRA, A., LÓPEZ-ALONSO, V., CHEERAN, B. & SUPPA, A. 2020b. Solutions for managing variability in non-invasive brain stimulation studies. *Neuroscience Letters,* 719.

HAMADA, M., HANAJIMA, R., TERAO, Y., ARAI, N., FURUBAYASHI, T., INOMATA-TERADA, S., YUGETA, A., MATSUMOTO, H., SHIROTA, Y. & UGAWA, Y. 2007. Quadro-pulse stimulation is more effective than paired-pulse stimulation for plasticity induction of the human motor cortex. *Clin Neurophysiol,* 118**,** 2672-82.

HAMADA, M., MURASE, N., HASAN, A., BALARATNAM, M. & ROTHWELL, J. C. 2013. The role of interneuron networks in driving human motor cortical plasticity. *Cereb Cortex,* 23**,** 1593-605.

HAMADA, M., TERAO, Y., HANAJIMA, R., SHIROTA, Y., NAKATANI-ENOMOTO, S., FURUBAYASHI, T., MATSUMOTO, H. & UGAWA, Y. 2008. Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. *J Physiol,* 586**,** 3927-47.

HANLEY, J. G. 2018. The Regulation of AMPA Receptor Endocytosis by Dynamic Protein-Protein Interactions. *Front Cell Neurosci,* 12**,** 362.

HANSEN, T., SKYTTHE, A., STENAGER, E., PETERSEN, H. C., BRONNUM-HANSEN, H. & KYVIK, K. O. 2005. Concordance for multiple sclerosis in Danish twins: an update of a nationwide study. *Mult Scler,* 11**,** 504-10.

HARDING-FORRESTER, S., ROOS, I., NGUYEN, A. L., MALPAS, C. B., DIOUF, I., MORADI, N., SHARMIN, S., IZQUIERDO, G., EICHAU, S., PATTI, F., HORAKOVA, D., HAVRDOVA, E. K., PRAT, A., GIRARD, M., DUQUETTE, P., MAISON, F. G., ONOFRJ, M., LUGARESI, A., GRAMMOND, P., OZAKBAS, S., AMATO, M. P., GERLACH, O., SOLA, P., FERRARO, D., BUZZARD, K., SKIBINA, O., LECHNER-SCOTT, J., ALROUGHANI, R., BOZ, C., VAN PESCH, V., CARTECHINI, E., TERZI, M., MAIMONE, D., RAMO-TELLO, C., YAMOUT, B., KHOURY, S. J., LA SPITALERI, D., SA, M. J., BLANCO, Y., GRANELLA, F., SLEE, M., BUTLER, E., SIDHOM, Y., GOUIDER, R., BERGAMASCHI, R., KARABUDAK, R., AMPAPA, R., SANCHEZ-MENOYO, J. L., PREVOST, J., CASTILLO-TRIVINO, T., MCCOMBE, P. A., MACDONELL, R., LAUREYS, G., VAN HIJFTE, L., OH, J., ALTINTAS, A., DE GANS, K., TURKOGLU, R., VAN DER WALT, A., BUTZKUEVEN, H., VUCIC, S., BARNETT, M., CRISTIANO, E., HODGKINSON, S., IULIANO, G., KAPPOS, L., KUHLE, J., SHAYGANNEJAD,

V., SOYSAL, A., WEINSTOCK-GUTTMAN, B., VAN WIJMEERSCH, B., KALINCIK, T. & INVESTIGATORS, M. 2023. Disability accrual in primary and secondary progressive multiple sclerosis. *Journal of Neurology Neurosurgery and Psychiatry,* 94**,** 707-717.

HAUSER, S. L. & CREE, B. A. C. 2020. Treatment of Multiple Sclerosis: A Review. *Am J Med,* 133**,** 1380-1390 e2.

HAUSER, S. L., DAWSON, D. M., LEHRICH, J. R., BEAL, M. F., KEVY, S. V., PROPPER, R. D., MILLS, J. A. & WEINER, H. L. 1983. Intensive immunosuppression in progressive multiple sclerosis. A randomized, three-arm study of high-dose intravenous cyclophosphamide, plasma exchange, and ACTH. *N Engl J Med,* 308**,** 173-80.

HERNANDEZ-PAVON, J. C., SCHNEIDER-GARCES, N., BEGNOCHE, J. P., MILLER, L. E. & RAIJ, T. 2023a. Targeted Modulation of Human Brain Interregional Effective Connectivity With Spike-Timing Dependent Plasticity. *Neuromodulation,* 26**,** 745-754.

HERNANDEZ-PAVON, J. C., VENIERO, D., BERGMANN, T. O., BELARDINELLI, P., BORTOLETTO, M., CASAROTTO, S., CASULA, E. P., FARZAN, F., FECCHIO, M., JULKUNEN, P., KALLIONIEMI, E., LIOUMIS, P., METSOMAA, J., MINIUSSI, C., MUTANEN, T. P., ROCCHI, L., ROGASCH, N. C., SHAFI, M. M., SIEBNER, H. R., THUT, G., ZRENNER, C., ZIEMANN, U. & ILMONIEMI, R. J. 2023b. TMS combined with EEG: Recommendations and open issues for data collection and analysis. *Brain Stimul,* 16**,** 567-593.

HERRMANN-LINGEN, C., BUSS, U. & SNAITH, R. 2018. *Hospital anxiety and depression scale - Deutsche Version. Deutsche Adaption der Hospital Anxiety and Depression Scale (HADS) von R.P. Snaith und A.S. Zigmond.,* Bern, Hogrefe.

HERVAULT, M., BALTO, J. M., HUBBARD, E. A. & MOTL, R. W. 2017. Reliability, precision, and clinically important change of the Nine-Hole Peg Test in individuals with multiple sclerosis. *Int J Rehabil Res,* 40**,** 91-93.

HOBART, J., LAMPING, D., FITZPATRICK, R., RIAZI, A. & THOMPSON, A. 2001. The Multiple Sclerosis Impact Scale (MSIS-29) - A new patient-based outcome measure. *Brain,* 124**,** 962-973.

HOCHBERG, Y. 1988. A Sharper Bonferroni Procedure for Multiple Tests of Significance. *Biometrika,* 75**,** 800-802.

HONARMAND, K. & FEINSTEIN, A. 2009. Validation of the Hospital Anxiety and Depression Scale for use with multiple sclerosis patients. *Multiple Sclerosis Journal,* 15**,** 1518-1524.

HOUSLEY, W. J., PITT, D. & HAFLER, D. A. 2015. Biomarkers in multiple sclerosis. *Clin Immunol,* 161**,** 51-8.

HUANG, W. C., LIN, H. C., YANG, Y. H., HSU, C. W., CHEN, N. C., TSAI, W. C., CHENG, B. C. & TSAI, N. W. 2022. Neutrophil-to-lymphocyte ratio and monocyte-to-lymphocyte ratio are associated with a 2-year relapse in patients with multiple sclerosis. *Mult Scler Relat Disord,* 58**,** 103514.

HUANG, Y. Z., EDWARDS, M. J., ROUNIS, E., BHATIA, K. P. & ROTHWELL, J. C. 2005. Theta burst stimulation of the human motor cortex. *Neuron,* 45**,** 201-6.

HUMAIDAN, D., XU, J., KIRCHHOFF, M., ROMANI, G. L., ILMONIEMI, R. J. & ZIEMANN, U. 2024. Towards real-time EEG-TMS modulation of brain state in a closed-loop approach. *Clin Neurophysiol,* 158**,** 212-217.

HUNTER, S. F. 2016. Overview and diagnosis of multiple sclerosis. *Am J Manag Care,* 22**,** s141-50.

IYER, M. B., SCHLEPER, N. & WASSERMANN, E. M. 2003. Priming stimulation enhances the depressant effect of low-frequency repetitive transcranial magnetic stimulation. *J Neurosci,* 23**,** 10867-72.

JACOBS, B. M., NOYCE, A. J., BESTWICK, J., BELETE, D., GIOVANNONI, G. & DOBSON, R. 2021. Gene-Environment Interactions in Multiple Sclerosis: A UK Biobank Study. *Neurol Neuroimmunol Neuroinflamm,* 8.

JAKIMOVSKI, D., KAVAK, K. S., VAUGHN, C. B., GOODMAN, A. D., COYLE, P. K., KRUPP, L., GOTTESMAN, M., EDWARDS, K. R., LENIHAN, M., PEREL, A., ZIVADINOV, R., WEINSTOCK-GUTTMAN, B. & NEW YORK STATE MULTIPLE SCLEROSIS, C. 2022. Discontinuation of disease modifying therapies is associated with disability progression regardless of prior stable disease and age. *Mult Scler Relat Disord,* 57**,** 103406.

JANG, S. H., CHO, S. H., KIM, Y. H., YOU, S. H., KIM, S. H., KIM, O., YANG, D. S. & SON, S. 2005. Motor recovery mechanism of diffuse axonal injury: a combined study of transcranial magnetic stimulation and functional MRI. *Restor Neurol Neurosci,* 23**,** 51-6.

JANNATI, A., OBERMAN, L. M., ROTENBERG, A. & PASCUAL-LEONE, A. 2023. Assessing the mechanisms of brain plasticity by transcranial magnetic stimulation. *Neuropsychopharmacology,* 48**,** 191-208.

JANSSENS, S. E. W. & SACK, A. T. 2021. Spontaneous Fluctuations in Oscillatory Brain State Cause Differences in Transcranial Magnetic Stimulation Effects Within and Between Individuals. *Frontiers in Human Neuroscience,* 15.

JOISTEN, N., PROSCHINGER, S., RADEMACHER, A., SCHENK, A., BLOCH, W., WARNKE, C., GONZENBACH, R., KOOL, J., BANSI, J. & ZIMMER, P. 2021. High-intensity interval training reduces neutrophil-to-lymphocyte ratio in persons with multiple sclerosis during inpatient rehabilitation. *Mult Scler,* 27**,** 1136-1139.

JULKUNEN, P., SAISANEN, L., DANNER, N., NISKANEN, E., HUKKANEN, T., MERVAALA, E. & KONONEN, M. 2009. Comparison of navigated and non-navigated transcranial magnetic stimulation for motor cortex mapping, motor threshold and motor evoked potentials. *Neuroimage,* 44**,** 790-5.

JÜRGENS, T., JAFARI, M., KREUTZFELDT, M., BAHN, E., BRUCK, W., KERSCHENSTEINER, M. & MERKLER, D. 2016. Reconstruction of single cortical projection neurons reveals primary spine loss in multiple sclerosis. *Brain,* 139**,** 39-46.

KAARRE, O., KALLIONIEMI, E., KONONEN, M., TOLMUNEN, T., KEKKONEN, V., KIVIMAKI, P., HEIKKINEN, N., FERRERI, F., LAUKKANEN, E. & MAATTA, S. 2018. Heavy alcohol use in adolescence is associated with altered cortical activity: a combined TMS-EEG study. *Addiction Biology,* 23**,** 268-280.

KAELIN-LANG, A. 2007. Motorisch evozierte Potenziale — Eine Einführung. *In:* SIEBNER, H. R. & ZIEMANN, U. (eds.) *Das TMS-Buch: Handbuch der transkraniellen Magnetstimulation.* Berlin, Heidelberg: Springer Berlin Heidelberg.

KAHL, C. K., GIUFFRE, A., WRIGHTSON, J. G., ZEWDIE, E., CONDLIFFE, E. G., MACMASTER, F. P. & KIRTON, A. 2023. Reliability of active robotic neuro-navigated transcranial magnetic stimulation motor maps. *Experimental Brain Research,* 241**,** 355-364.

KALLAUR, A. P., OLIVEIRA, S. R., SIMAO, A. N. C., ALFIERI, D. F., FLAUZINO, T., LOPES, J., DE CARVALHO JENNINGS PEREIRA, W. L., DE MELECK PROENCA, C., BORELLI, S. D., KAIMEN-MACIEL, D. R., MAES, M. & REICHE, E. M. V. 2017. Cytokine Profile in Patients with Progressive Multiple Sclerosis and Its Association with Disease Progression and Disability. *Mol Neurobiol,* 54**,** 2950-2960.

KARABANOV, A., ZIEMANN, U., HAMADA, M., GEORGE, M. S., QUARTARONE, A., CLASSEN, J., MASSIMINI, M., ROTHWELL, J. & SIEBNER, H. R. 2015. Consensus Paper: Probing Homeostatic Plasticity of Human Cortex With Non-invasive Transcranial Brain Stimulation. *Brain Stimulation,* 8**,** 993-1006.

KARABANOV, A. N., MADSEN, K. H., KROHNE, L. G. & SIEBNER, H. R. 2021. Does pericentral mu-rhythm "power" corticomotor excitability?- A matter of EEG perspective. *Brain Stimulation,* 14**,** 713-722.
KARIMI, N., ASHOURIZADEH, H., AKBARZADEH PASHA, B., HAGHSHOMAR, M., JOUZDANI, T., SHOBEIRI, P., TEIXEIRA, A. L. & REZAEI, N. 2022. Blood levels of brain-derived neurotrophic factor (BDNF) in people with multiple sclerosis (MS): A systematic review and meta-analysis. *Mult Scler Relat Disord,* 65**,** 103984.
KELLOR, M. 1971. Hand strength and dexterity. *American Journal of Occupational Therapy,* 25**,** 77-83.
KIM, H. Y. 2015. Statistical notes for clinical researchers: post-hoc multiple comparisons. *Restor Dent Endod,* 40**,** 172-6.
KIM, J. H. 2019. Multicollinearity and misleading statistical results. *Korean J Anesthesiol,* 72**,** 558-569.
KISHORE, A., JOSEPH, T., VELAYUDHAN, B., POPA, T. & MEUNIER, S. 2012. Early, severe and bilateral loss of LTP and LTD-like plasticity in motor cortex (M1) in de novo Parkinson's disease. *Clin Neurophysiol,* 123**,** 822-8.
KLOMJAI, W., KATZ, R. & LACKMY-VALLEE, A. 2015. Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Ann Phys Rehabil Med,* 58**,** 208-213.
KOCH-HENRIKSEN, N. & SORENSEN, P. S. 2010. The changing demographic pattern of multiple sclerosis epidemiology. *Lancet Neurol,* 9**,** 520-32.
KOPONEN, L. M., NIEMINEN, J. O. & ILMONIEMI, R. J. 2018. Multi-locus transcranial magnetic stimulation-theory and implementation. *Brain Stimul,* 11**,** 849-855.
KSIAZEK-WINIAREK, D. J., SZPAKOWSKI, P. & GLABINSKI, A. 2015. Neural Plasticity in Multiple Sclerosis: The Functional and Molecular Background. *Neural Plasticity,* 2015.
KUHN, A. A., GROSSE, P., HOLTZ, K., BROWN, P., MEYER, B. U. & KUPSCH, A. 2004. Patterns of abnormal motor cortex excitability in atypical parkinsonian syndromes. *Clinical Neurophysiology,* 115**,** 1786-1795.
KUNZE, R., FISCHER, S., MARTI, H. H. & PREISSNER, K. T. 2023. Brain alarm by self-extracellular nucleic acids: from neuroinflammation to neurodegeneration. *J Biomed Sci,* 30**,** 64.
KURTZKE, J. F. 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology,* 33**,** 1444-52.
KUUSISTO, H., KAPRIO, J., KINNUNEN, E., LUUKKAALA, T., KOSKENVUO, M. & ELOVAARA, I. 2008. Concordance and heritability of multiple sclerosis in Finland: study on a nationwide series of twins. *Eur J Neurol,* 15**,** 1106-10.
LANG, N., HARMS, J., WEYH, T., LEMON, R. N., PAULUS, W., ROTHWELL, J. C. & SIEBNER, H. R. 2006. Stimulus intensity and coil characteristics influence the efficacy of rTMS to suppress cortical excitability. *Clin Neurophysiol,* 117**,** 2292-301.
LANG, N., ROTHKEGEL, H., REIBER, H., HASAN, A., SUESKE, E., TERGAU, F., EHRENREICH, H., WUTTKE, W. & PAULUS, W. 2011. Circadian modulation of GABA-mediated cortical inhibition. *Cereb Cortex,* 21**,** 2299-306.
LASSMANN, H. 2014. Mechanisms of white matter damage in multiple sclerosis. *Glia,* 62**,** 1816-30.
LASSMANN, H. 2018. Pathogenic Mechanisms Associated With Different Clinical Courses of Multiple Sclerosis. *Front Immunol,* 9**,** 3116.
LASSMANN, H., VAN HORSSEN, J. & MAHAD, D. 2012. Progressive multiple sclerosis: pathology and pathogenesis. *Nat Rev Neurol,* 8**,** 647-56.
LEARMONTH, Y. C., PAUL, L., MCFADYEN, A. K., MATTISON, P. & MILLER, L. 2012. Reliability and clinical significance of mobility and balance assessments in multiple sclerosis. *Int J Rehabil Res,* 35**,** 69-74.

LEITE, J., MORALES-QUEZADA, L., CARVALHO, S., THIBAUT, A., DORUK, D., CHEN, C. F., SCHACHTER, S. C., ROTENBERG, A. & FREGNI, F. 2017. Surface EEG-Transcranial Direct Current Stimulation (tDCS) Closed-Loop System. *International Journal of Neural Systems,* 27.

LENTH, R. V. 2023. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.9.0 ed.

LERAY, E., MOREAU, T., FROMONT, A. & EDAN, G. 2016. Epidemiology of multiple sclerosis. *Rev Neurol (Paris),* 172**,** 3-13.

LERAY, E., VUKUSIC, S., DEBOUVERIE, M., CLANET, M., BROCHET, B., DE SEZE, J., ZEPHIR, H., DEFER, G., LEBRUN-FRENAY, C., MOREAU, T., CLAVELOU, P., PELLETIER, J., BERGER, E., CABRE, P., CAMDESSANCHE, J. P., KALSON-RAY, S., CONFAVREUX, C. & EDAN, G. 2015. Excess Mortality in Patients with Multiple Sclerosis Starts at 20 Years from Clinical Onset: Data from a Large-Scale French Observational Study. *PLoS One,* 10**,** e0132033.

LEVITE, M. 2017. Glutamate, T cells and multiple sclerosis. *Journal of Neural Transmission,* 124**,** 775-798.

LEWSEY, J. D., GARDINER, W. P. & GETTINBY, G. 1997. A study of simple unbalanced factorial designs that use Type II and Type III sums of squares. *Communications in Statistics-Simulation and Computation,* 26**,** 1315-1328.

LI, Q., NAVAKKODE, S., ROTHKEGEL, M., SOONG, T. W., SAJIKUMAR, S. & KORTE, M. 2017. Metaplasticity mechanisms restore plasticity and associativity in an animal model of Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America,* 114**,** 5527-5532.

LIU, X., WU, Z., HAYASHI, Y. & NAKANISHI, H. 2012. Age-Dependent Neuroinflammatory Responses and Deficits in Long-Term Potentiation in the Hippocampus during Systemic Inflammation. *Neuroscience,* 216**,** 133-142.

LLANOS, C., RODRIGUEZ, M., RODRIGUEZ-SABATE, C., MORALES, I. & SABATE, M. 2013. Mu-rhythm changes during the planning of motor and motor imagery actions. *Neuropsychologia,* 51**,** 1019-26.

LOITFELDER, M., FAZEKAS, F., PETROVIC, K., FUCHS, S., ROPELE, S., WALLNER-BLAZEK, M., JEHNA, M., ASPECK, E., KHALIL, M., SCHMIDT, R., NEUPER, C. & ENZINGER, C. 2011. Reorganization in cognitive networks with progression of multiple sclerosis Insights from fMRI. *Neurology,* 76**,** 526-533.

LU, W., MAN, H., JU, W., TRIMBLE, W. S., MACDONALD, J. F. & WANG, Y. T. 2001. Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron,* 29**,** 243-54.

LUBLIN, F. D. 2014. New multiple sclerosis phenotypic classification. *Eur Neurol,* 72 Suppl 1**,** 1-5.

LUBLIN, F. D., COETZEE, T., COHEN, J. A., MARRIE, R. A., THOMPSON, A. J. & INTERNATIONAL ADVISORY COMMITTEE ON CLINICAL TRIALS IN, M. S. 2020. The 2013 clinical course descriptors for multiple sclerosis: A clarification. *Neurology,* 94**,** 1088-1092.

LUBLIN, F. D., HARING, D. A., GANJGAHI, H., OCAMPO, A., HATAMI, F., CUKLINA, J., AARDEN, P., DAHLKE, F., ARNOLD, D. L., WIENDL, H., CHITNIS, T., NICHOLS, T. E., KIESEIER, B. C. & BERMEL, R. A. 2022. How patients with multiple sclerosis acquire disability. *Brain,* 145**,** 3147-3161.

LUBLIN, F. D. & REINGOLD, S. C. 1996. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology,* 46**,** 907-11.

LUBLIN, F. D., REINGOLD, S. C., COHEN, J. A., CUTTER, G. R., SORENSEN, P. S., THOMPSON, A. J., WOLINSKY, J. S., BALCER, L. J., BANWELL, B.,

BARKHOF, F., BEBO, B., JR., CALABRESI, P. A., CLANET, M., COMI, G., FOX, R. J., FREEDMAN, M. S., GOODMAN, A. D., INGLESE, M., KAPPOS, L., KIESEIER, B. C., LINCOLN, J. A., LUBETZKI, C., MILLER, A. E., MONTALBAN, X., O'CONNOR, P. W., PETKAU, J., POZZILLI, C., RUDICK, R. A., SORMANI, M. P., STUVE, O., WAUBANT, E. & POLMAN, C. H. 2014. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology,* 83**,** 278-86.

LUCHETTI, S., FRANSEN, N. L., VAN EDEN, C. G., RAMAGLIA, V., MASON, M. & HUITINGA, I. 2018. Progressive multiple sclerosis patients show substantial lesion activity that correlates with clinical disease severity and sex: a retrospective autopsy cohort analysis. *Acta Neuropathol,* 135**,** 511-528.

LUCK, T., RIEDEL-HELLER, S., WIESE, B., STEIN, J., WEYERER, S., WERLE, J., KADUSZKIEWICZ, H., WAGNER, M., MÖSCH, E. & ZIMMERMANN, T. 2009. CERAD-NP-testbatterie: alters-, geschlechts-und bildungsspezifische normen ausgewählter subtests. *Zeitschrift für Gerontologie und Geriatrie,* 42**,** 372-384.

LUSCHER, C. & MALENKA, R. C. 2012. NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harb Perspect Biol,* 4.

MA, K., HAMADA, M., DI LAZZARO, V., HAND, B., GUERRA, A., OPIE, G. M. & GOETZ, S. M. 2023. Correlating active and resting motor thresholds for transcranial magnetic stimulation through a matching model. *Brain Stimulation,* 16**,** 1686-1688.

MACHADO, R., LIMA, C., D'ALMEIDA, O. C., AFONSO, A., MACARIO, C., CASTELO-BRANCO, M., SOUSA, L., SANTANA, I. & BATISTA, S. 2021. Protective effects of cognitive and brain reserve in multiple sclerosis: Differential roles on social cognition and 'classic cognition'. *Mult Scler Relat Disord,* 48**,** 102716.

MACREZ, R., STYS, P. K., VIVIEN, D., LIPTON, S. A. & DOCAGNE, F. 2016. Mechanisms of glutamate toxicity in multiple sclerosis: biomarker and therapeutic opportunities. *Lancet Neurol,* 15**,** 1089-102.

MAHAD, D. H., TRAPP, B. D. & LASSMANN, H. 2015. Pathological mechanisms in progressive multiple sclerosis. *Lancet Neurol,* 14**,** 183-93.

MALAY, S. & CHUNG, K. C. 2012. The Choice of Controls for Providing Validity and Evidence in Clinical Research. *Plastic and Reconstructive Surgery,* 130**,** 959-965.

MALLOY, S., GENOVA, H., CHIARAVALLOTI, N., DELUCA, J., HOLTZHEIMER, P. & WYLIE, G. R. 2021. Cognitive fatigue in traumatic brain injury: a pilot study comparing state and trait fatigue. *Brain Inj,* 35**,** 1254-1258.

MANOGARAN, P., VAVASOUR, I., BORICH, M., KOLIND, S. H., LANGE, A. P., RAUSCHER, A., BOYD, L., LI, D. K. & TRABOULSEE, A. 2016. Corticospinal tract integrity measured using transcranial magnetic stimulation and magnetic resonance imaging in neuromyelitis optica and multiple sclerosis. *Mult Scler,* 22**,** 43-50.

MANOUCHEHRINIA, A., WESTERLIND, H., KINGWELL, E., ZHU, F., CARRUTHERS, R., RAMANUJAM, R., BAN, M., GLASER, A., SAWCER, S., TREMLETT, H. & HILLERT, J. 2017. Age Related Multiple Sclerosis Severity Score: Disability ranked by age. *Mult Scler,* 23**,** 1938-1946.

MARTEL, M. & GLOVER, S. 2023. TMS over dorsolateral prefrontal cortex affects the timing of motor imagery but not overt action: Further support for the motor-cognitive model. *Behav Brain Res,* 437**,** 114125.

MARTIN-RODRIGUEZ, J. F., RUIZ-VEGUILLA, M., DE TOLEDO, P. A., AIZPURUA-OLAIZOLA, O., ZARANDONA, I., CANAL-RIVERO, M., RODRIGUEZ-BAENA, A. & MIR, P. 2021. Impaired motor cortical plasticity associated with cannabis use disorder in young adults. *Addiction Biology,* 26.

MASSEY, P. V. & BASHIR, Z. I. 2007. Long-term depression: multiple forms and implications for brain function. *Trends Neurosci,* 30**,** 176-84.

MATHIOWETZ, V., WEBER, K., KASHMAN, N. & VOLLAND, G. 1985. Adult Norms for the Nine Hole Peg Test of Finger Dexterity. *The Occupational Therapy Journal of Research,* 5**,** 24-38.
MATSUMOTO, H. & UGAWA, Y. 2020. Quadripulse stimulation (QPS). *Exp Brain Res,* 238**,** 1619-1625.
MAYEUX, R. 2004. Biomarkers: potential uses and limitations. *NeuroRx,* 1**,** 182-8.
MCKAY, W. B., STOKIC, D. S. & DIMITRIJEVIC, M. R. 1997. Assessment of corticospinal function in spinal cord injury using transcranial motor cortex stimulation: a review. *J Neurotrauma,* 14**,** 539-48.
MCNEISH, D. & STAPLETON, L. M. 2016. Modeling Clustered Data with Very Few Clusters. *Multivariate Behavioral Research,* 51**,** 495-518.
MEAD, G., BERNHARDT, J. & KWAKKEL, G. 2012. Stroke: physical fitness, exercise, and fatigue. *Stroke Res Treat,* 2012**,** 632531.
MEHRPOUR, M., AKHOUNDI, F. H., DELGOSHA, M., KEYVANI, H., MOTAMED, M. R., SHEIBANI, B. & MEYSAMIE, A. 2015. Increased Serum Brain-derived Neurotrophic Factor in Multiple Sclerosis Patients on Interferon-β and Its Impact on Functional Abilities. *Neurologist,* 20**,** 57-60.
MEINCKE, J., HEWITT, M., BATSIKADZE, G. & LIEBETANZ, D. 2016. Automated TMS hotspot-hunting using a closed loop threshold-based algorithm. *Neuroimage,* 124**,** 509-517.
MEMORY CLINIC. n.d. *cerad-plus - leitfaden zur berechnung der ausbildungsjahre* [Online]. Available: https://www.memoryclinic.ch/fileadmin/user_upload/Memory_Clinic/CERAD-Plus/CERAD-Plus_Leitfaden_zur_Berechnung_der_Ausbildungsjahre.pdf [Accessed].
MENARDI, A., OZDEMIR, R. A., MOMI, D., TADAYON, E., BOUCHER, P., VALLESI, A., PASCUAL-LEONE, A., SHAFI, M. M. & SANTARNECCHI, E. 2022. Effect of group-based vs individualized stimulation site selection on reliability of network-targeted TMS. *Neuroimage,* 264**,** 119714.
MEYER-MOOCK, S., FENG, Y. S., MAEURER, M., DIPPEL, F. W. & KOHLMANN, T. 2014. Systematic literature review and validity evaluation of the Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Functional Composite (MSFC) in patients with multiple sclerosis. *BMC Neurol,* 14**,** 58.
MINKOVA, L., PETER, J., ABDULKADIR, A., SCHUMACHER, L. V., KALLER, C. P., NISSEN, C., KLOPPEL, S. & LAHR, J. 2019. Determinants of Inter-Individual Variability in Corticomotor Excitability Induced by Paired Associative Stimulation. *Front Neurosci,* 13**,** 841.
MIRON, J. P., JODOIN, V. D., LESPÉRANCE, P. & BLUMBERGER, D. M. 2021. Repetitive transcranial magnetic stimulation for major depressive disorder: basic principles and future directions. *Therapeutic Advances in Psychopharmacology,* 11.
MIRRA, S. S., HEYMAN, A., MCKEEL, D., SUMI, S., CRAIN, B. J., BROWNLEE, L., VOGEL, F., HUGHES, J., VAN BELLE, G. & BERG, L. 1991. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD): Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology,* 41**,** 479-479.
MOLLISON, D., SELLAR, R., BASTIN, M., MOLLISON, D., CHANDRAN, S., WARDLAW, J. & CONNICK, P. 2017. The clinico-radiological paradox of cognitive function and MRI burden of white matter lesions in people with multiple sclerosis: A systematic review and meta-analysis. *PLoS One,* 12**,** e0177727.
MORI, F., KUSAYANAGI, H., BUTTARI, F., CENTINI, B., MONTELEONE, F., NICOLETTI, C. G., BERNARDI, G., DI CANTOGNO, E. V., MARCIANI, M. G. & CENTONZE, D. 2012. Early treatment with high-dose interferon beta-1a reverses cognitive and cortical plasticity de fi cits in multiple sclerosis. *Funct Neurol,* 27**,** 163-8.

MORI, F., KUSAYANAGI, H., NICOLETTI, C. G., WEISS, S., MARCIANI, M. G. & CENTONZE, D. 2014a. Cortical plasticity predicts recovery from relapse in multiple sclerosis. *Mult Scler,* 20**,** 451-7.

MORI, F., NICOLETTI, C. G., ROSSI, S., MOTTA, C., KUSAYANAGI, H., BERGAMI, A., STUDER, V., BUTTARI, F., BARBIERI, F., WEISS, S., NISTICO, R., MARTINO, G., FURLAN, R. & CENTONZE, D. 2014b. Growth factors and synaptic plasticity in relapsing-remitting multiple sclerosis. *Neuromolecular Med,* 16**,** 490-8.

MORI, F., ROSSI, S., PICCININ, S., MOTTA, C., MANGO, D., KUSAYANAGI, H., BERGAMI, A., STUDER, V., NICOLETTI, C. G., BUTTARI, F., BARBIERI, F., MERCURI, N. B., MARTINO, G., FURLAN, R., NISTICO, R. & CENTONZE, D. 2013. Synaptic plasticity and PDGF signaling defects underlie clinical progression in multiple sclerosis. *J Neurosci,* 33**,** 19112-9.

MORRIS, J. C., HEYMAN, A., MOHS, R. C., HUGHES, J., VAN BELLE, G., FILLENBAUM, G., MELLITS, E. & CLARK, C. 1989. The consortium to establish a registry for Alzheimer's disease (CERAD): I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology*.

MORRIS, S. B. 2008. Estimating effect sizes from pretest-posttest-control group designs. *Organizational Research Methods,* 11**,** 364-386.

MOTL, R. W., COHEN, J. A., BENEDICT, R., PHILLIPS, G., LAROCCA, N., HUDSON, L. D., RUDICK, R. & OUTCOME, M. S. 2017. Validity of the timed 25-foot walk as an ambulatory performance outcome measure for multiple sclerosis. *Multiple Sclerosis Journal,* 23**,** 704-710.

MOUTSIANAS, L., JOSTINS, L., BEECHAM, A. H., DILTHEY, A. T., XIFARA, D. K., BAN, M., SHAH, T. S., PATSOPOULOS, N. A., ALFREDSSON, L., ANDERSON, C. A., ATTFIELD, K. E., BARANZINI, S. E., BARRETT, J., BINDER, T. M. C., BOOTH, D., BUCK, D., CELIUS, E. G., COTSAPAS, C., D'ALFONSO, S., DENDROU, C. A., DONNELLY, P., DUBOIS, B., FONTAINE, B., FUGGER, L., GORIS, A., GOURRAUD, P. A., GRAETZ, C., HEMMER, B., HILLERT, J., INTERNATIONAL, I. B. D. G. C., KOCKUM, I., LESLIE, S., LILL, C. M., MARTINELLI-BONESCHI, F., OKSENBERG, J. R., OLSSON, T., OTURAI, A., SAARELA, J., SONDERGAARD, H. B., SPURKLAND, A., TAYLOR, B., WINKELMANN, J., ZIPP, F., HAINES, J. L., PERICAK-VANCE, M. A., SPENCER, C. C. A., STEWART, G., HAFLER, D. A., IVINSON, A. J., HARBO, H. F., HAUSER, S. L., DE JAGER, P. L., COMPSTON, A., MCCAULEY, J. L., SAWCER, S. & MCVEAN, G. 2015. Class II HLA interactions modulate genetic risk for multiple sclerosis. *Nat Genet,* 47**,** 1107-1113.

MUELLBACHER, W., FACCHINI, S., BOROOJERDI, B. & HALLETT, M. 2000. Changes in motor cortex excitability during ipsilateral hand muscle activation in humans. *Clin Neurophysiol,* 111**,** 344-9.

MÜLLER-DAHLHAUS, F. & ZIEMANN, U. 2015. Metaplasticity in Human Cortex. *Neuroscientist,* 21**,** 185-202.

MÜLLER-DAHLHAUS, F., ZIEMANN, U. & CLASSEN, J. 2010. Plasticity resembling spike-timing dependent synaptic plasticity: the evidence in human cortex. *Front Synaptic Neurosci,* 2**,** 34.

MULLINEAUX, D. R. & WHEAT, J. 2017. Research methods: sample size and variability effects on statistical power. *In:* PAYTON, C. J. & BURDEN, A. (eds.) *Biomechanical Evaluation of Movement in Sport and Exercise.* 2 ed.: Routledge.

MURPHY, O. C., CALABRESI, P. A. & SAIDHA, S. 2023. Trans-synaptic degeneration as a mechanism of neurodegeneration in multiple sclerosis. *Neural Regeneration Research,* 18**,** 2682-2684.

MUSELLA, A., GENTILE, A., RIZZO, F. R., DE VITO, F., FRESEGNA, D., BULLITTA, S., VANNI, V., GUADALUPI, L., BASSI, M. S., BUTTARI, F., CENTONZE, D. &

MANDOLESI, G. 2018. Interplay Between Age and Neuroinflammation in Multiple Sclerosis: Effects on Motor and Cognitive Functions. *Frontiers in Aging Neuroscience,* 10.
NAKAMURA, K., GROISS, S. J., HAMADA, M., ENOMOTO, H., KADOWAKI, S., ABE, M., MURAKAMI, T., WIRATMAN, W., CHANG, F., KOBAYASHI, S., HANAJIMA, R., TERAO, Y. & UGAWA, Y. 2016. Variability in Response to Quadripulse Stimulation of the Motor Cortex. *Brain Stimul,* 9**,** 859-866.
NAZERI, M., BAZRAFSHAN, H. & FOROUGHI, A. A. 2022. Serum inflammatory markers in patients with multiple sclerosis and their association with clinical manifestations and MRI findings. *Acta Neurologica Belgica,* 122**,** 1187-1193.
NICOLETTI, C. G., MONTELEONE, F., MARFIA, G. A., USIELLO, A., BUTTARI, F., CENTONZE, D. & MORI, F. 2020. Oral D-Aspartate enhances synaptic plasticity reserve in progressive multiple sclerosis. *Multiple Sclerosis Journal,* 26**,** 304-311.
NICOLL, R. A. & ROCHE, K. W. 2013. Long-term potentiation: peeling the onion. *Neuropharmacology,* 74**,** 18-22.
NIELSEN, N. M., MUNGER, K. L., STENAGER, E. & ASCHERIO, A. 2017. Author response: Neonatal vitamin D status and risk of multiple sclerosis: A population-based case-control study. *Neurology,* 89**,** 411.
NIELSEN, N. M., WESTERGAARD, T., ROSTGAARD, K., FRISCH, M., HJALGRIM, H., WOHLFAHRT, J., KOCH-HENRIKSEN, N. & MELBYE, M. 2005. Familial risk of multiple sclerosis: a nationwide cohort study. *Am J Epidemiol,* 162**,** 774-8.
NOREIKA, V., KAMKE, M. R., CANALES-JOHNSON, A., CHENNU, S., BEKINSCHTEIN, T. A. & MATTINGLEY, J. B. 2020. Alertness fluctuations when performing a task modulate cortical evoked responses to transcranial magnetic stimulation. *Neuroimage,* 223**,** 117305.
NOSEWORTHY, J. H., LUCCHINETTI, C., RODRIGUEZ, M. & WEINSHENKER, B. G. 2000. Multiple sclerosis. *N Engl J Med,* 343**,** 938-52.
OLSSON, T., BARCELLOS, L. F. & ALFREDSSON, L. 2017. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. *Nat Rev Neurol,* 13**,** 25-36.
PANDIS, N. 2016. The chi-square test. *Am J Orthod Dentofacial Orthop,* 150**,** 898-899.
PANTANO, P., MAINERO, C. & CARAMIA, F. 2006. Functional brain reorganization in multiple sclerosis: Evidence from fMRI studies. *Journal of Neuroimaging,* 16**,** 104-114.
PAPIRI, G., D'ANDREAMATTEO, G., CACCHIO, G., ALIA, S., SILVESTRINI, M., PACI, C., LUZZI, S. & VIGNINI, A. 2023. Multiple Sclerosis: Inflammatory and Neuroglial Aspects. *Curr Issues Mol Biol,* 45**,** 1443-1470.
PATANI, R., BALARATNAM, M., VORA, A. & REYNOLDS, R. 2007. Remyelination can be extensive in multiple sclerosis despite a long disease course. *Neuropathol Appl Neurobiol,* 33**,** 277-87.
PAULHUS, D. L., ROBINS, R. W., TRZESNIEWSKI, K. H. & TRACY, J. L. 2004. Two Replicable Suppressor Situations in Personality Research. *Multivariate Behav Res,* 39**,** 303-28.
PELLEGRINI, M., ZOGHI, M. & JABERZADEH, S. 2018. Biological and anatomical factors influencing interindividual variability to noninvasive brain stimulation of the primary motor cortex: a systematic review and meta-analysis. *Reviews in the Neurosciences,* 29**,** 199-222.
PENNER, I. K. 2023. Clinical Assessment Tools for Fatigue. *Fatigue in Multiple Sclerosis: Background, Clinic, Diagnostic, Therapy.* Cham: Springer International Publishing.
PENNER, I. K., RASELLI, C., STOCKLIN, M., OPWIS, K., KAPPOS, L. & CALABRESE, P. 2009. The Fatigue Scale for Motor and Cognitive Functions (FSMC): validation of a new instrument to assess multiple sclerosis-related fatigue. *Mult Scler,* 15**,** 1509-17.

PÉRAN, P., NEMMI, F., DUTILLEUL, C., FINAMORE, L., CARAVASSO, C. F., TROISI, E., IOSA, M., SABATINI, U. & GRASSO, M. G. 2020. Neuroplasticity and brain reorganization associated with positive outcomes of multidisciplinary rehabilitation in progressive multiple sclerosis: A fMRI study. *Multiple Sclerosis and Related Disorders,* 42.

PETROVA, N., NUTMA, E., CARASSITI, D., RS NEWMAN, J., AMOR, S., ALTMANN, D. R., BAKER, D. & SCHMIERER, K. 2020. Synaptic Loss in Multiple Sclerosis Spinal Cord. *Ann Neurol,* 88**,** 619-625.

PINHEIRO, J., BATES, D. & R CORE TEAM 2023. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-163 ed.

PITCHER, J. B., OGSTON, K. M. & MILES, T. S. 2003. Age and sex differences in human motor cortex input-output characteristics. *J Physiol,* 546**,** 605-13.

PROCHNOW, N., GOLD, R. & HAGHIKIA, A. 2013. An electrophysiologic approach to quantify impaired synaptic transmission and plasticity in experimental autoimmune encephalomyelitis. *Journal of Neuroimmunology,* 264**,** 48-53.

PROSPERINI, L., PIATTELLA, M. C., GIANNÌ, C. & PANTANO, P. 2015. Functional and Structural Brain Plasticity Enhanced by Motor and Cognitive Rehabilitation in Multiple Sclerosis. *Neural Plasticity,* 2015.

R CORE TEAM 2023. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

RADHU, N., DE JESUS, D. R., RAVINDRAN, L. N., ZANJANI, A., FITZGERALD, P. B. & DASKALAKIS, Z. J. 2013. A meta-analysis of cortical inhibition and excitability using transcranial magnetic stimulation in psychiatric disorders. *Clinical Neurophysiology,* 124**,** 1309-1320.

RAGINIS-ZBOROWSKA, A., CHENG, I., PENDLETON, N., PAYTON, A., OLLIER, W., MICHOU, E. & HAMDY, S. 2019. Genetic influences on the variability of response to repetitive transcranial magnetic stimulation in human pharyngeal motor cortex. *Neurogastroenterology and Motility,* 31.

RAO, S. M., COGNITIVE FUNCTION STUDY GROUP OF THE NATIONAL MULTIPLE SCLEROSIS SOCIETY 1990. *A manual for the brief repeatable battery of neuropsychological tests in multiple sclerosis,* Milwaukee, Medical College of Wisconsin.

RAYMOND, C. R. 2007. LTP forms 1, 2 and 3: different mechanisms for the 'long' in long-term potentiation. *Trends in Neurosciences,* 30**,** 167-175.

RICHTER, L., TRILLENBERG, P., SCHWEIKARD, A. & SCHLAEFER, A. 2013. Stimulus intensity for hand held and robotic transcranial magnetic stimulation. *Brain Stimul,* 6**,** 315-21.

RIZZO, F. R., MUSELLA, A., DE VITO, F., FRESEGNA, D., BULLITTA, S., VANNI, V., GUADALUPI, L., STAMPANONI BASSI, M., BUTTARI, F., MANDOLESI, G., CENTONZE, D. & GENTILE, A. 2018. Tumor Necrosis Factor and Interleukin-1beta Modulate Synaptic Plasticity during Neuroinflammation. *Neural Plast,* 2018**,** 8430123.

ROMERO-PINEL, L., BAU, L., MATAS, E., LEON, I., MUNOZ-VENDRELL, A., ARROYO, P., MASUET-AUMATELL, C., MARTINEZ-YELAMOS, A. & MARTINEZ-YELAMOS, S. 2022. The age at onset of relapsing-remitting multiple sclerosis has increased over the last five decades. *Mult Scler Relat Disord,* 68**,** 104103.

ROMME CHRISTENSEN, J., KOMORI, M., VON ESSEN, M. R., RATZER, R., BORNSEN, L., BIELEKOVA, B. & SELLEBJERG, F. 2019. CSF inflammatory biomarkers responsive to treatment in progressive multiple sclerosis capture residual inflammation associated with axonal damage. *Mult Scler,* 25**,** 937-946.

ROOS, I., HUGHES, S., MCDONNELL, G., MALPAS, C. B., SHARMIN, S., BOZ, C., ALROUGHANI, R., OZAKBAS, S., BUZZARD, K., SKIBINA, O., VAN DER

WALT, A., BUTZKUEVEN, H., LECHNER-SCOTT, J., KUHLE, J., TERZI, M., LAUREYS, G., VAN HIJFTE, L., JOHN, N., GRAMMOND, P., GRAND'MAISON, F., SOYSAL, A., JENSEN, A. V., RASMUSSEN, P. V., SVENDSEN, K. B., BARZINJI, I., NIELSEN, H. H., SEJBAEK, T., PRAKASH, S., STILUND, M. L. M., WEGLEWSKI, A., ISSA, N. M., KANT, M., SELLEBJERG, F., GRAY, O., MAGYARI, M., KALINCIK, T. & GROUP, M. S. S. G. M. R. S. 2023. Rituximab vs Ocrelizumab in Relapsing-Remitting Multiple Sclerosis. *JAMA Neurol,* 80**,** 789-797.

ROSSI, S., FURLAN, R., DE CHIARA, V., MOTTA, C., STUDER, V., MORI, F., MUSELLA, A., BERGAMI, A., MUZIO, L., BERNARDI, G., BATTISTINI, L., MARTINO, G. & CENTONZE, D. 2012. Interleukin-1beta causes synaptic hyperexcitability in multiple sclerosis. *Ann Neurol,* 71**,** 76-83.

ROSSI, S., HALLETT, M., ROSSINI, P. M. & PASCUAL-LEONE, A. 2011. Screening questionnaire before TMS: an update. *Clin Neurophysiol,* 122**,** 1686.

ROSSI, S., MOTTA, C., STUDER, V., BARBIERI, F., BUTTARI, F., BERGAMI, A., SANCESARIO, G., BERNARDINI, S., DE ANGELIS, G., MARTINO, G., FURLAN, R. & CENTONZE, D. 2014. Tumor necrosis factor is elevated in progressive multiple sclerosis and causes excitotoxic neurodegeneration. *Mult Scler,* 20**,** 304-12.

ROTHWELL, J. C., HALLETT, M., BERARDELLI, A., EISEN, A., ROSSINI, P. & PAULUS, W. 1999. Magnetic stimulation: motor evoked potentials. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl,* 52**,** 97-103.

RUOHONEN, J. & KARHU, J. 2010. Navigated transcranial magnetic stimulation. *Neurophysiol Clin,* 40**,** 7-17.

RUSU, C. V., MURAKAMI, M., ZIEMANN, U. & TRIESCH, J. 2014. A model of TMS-induced I-waves in motor cortex. *Brain Stimul,* 7**,** 401-14.

SABEL, C. E., PEARSON, J. F., MASON, D. F., WILLOUGHBY, E., ABERNETHY, D. A. & TAYLOR, B. V. 2021. The latitude gradient for multiple sclerosis prevalence is established in the early life course. *Brain,* 144**,** 2038-2046.

SACHDEV, S., KISHORE, K., SINGH, L., LAMBA, D. S., HANS, R., DHAWAN, H. K., GROVER, S. & SHARMA, R. R. 2021. Exploration of COVID-19 related fears deterring from blood donation in India. *ISBT Sci Ser,* 16**,** 147-157.

SALE, M. V., RIDDING, M. C. & NORDSTROM, M. A. 2007. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. *Exp Brain Res,* 181**,** 615-26.

SARCHIELLI, P., GRECO, L., FLORIDI, A., FLORIDI, A. & GALLAI, V. 2003. Excitatory amino acids and multiple sclerosis: evidence from cerebrospinal fluid. *Arch Neurol,* 60**,** 1082-8.

SCHERER, P., BAUM, K., BAUER, H., GOHLER, H. & MILTENBURGER, C. 2004. [Normalization of the Brief Repeatable Battery of Neuropsychological tests (BRB-N) for German-speaking regions. Application in relapsing-remitting and secondary progressive multiple sclerosis patients]. *Nervenarzt,* 75**,** 984-90.

SHIBUYA, K., PARK, S. B., GEEVASINGA, N., HUYNH, W., SIMON, N. G., MENON, P., HOWELLS, J., VUCIC, S. & KIERNAN, M. C. 2016. Threshold tracking transcranial magnetic stimulation: Effects of age and gender on motor cortical function. *Clin Neurophysiol,* 127**,** 2355-61.

SHULGA, A., LIOUMIS, P., KIRVESKARI, E., SAVOLAINEN, S., MAKELA, J. P. & YLINEN, A. 2015. The use of F-response in defining interstimulus intervals appropriate for LTP-like plasticity induction in lower limb spinal paired associative stimulation. *Journal of Neuroscience Methods,* 242**,** 112-117.

SIGNORI, A., LORSCHEIDER, J., VUKUSIC, S., TROJANO, M., IAFFALDANO, P., HILLERT, J., HYDE, R., PELLEGRINI, F., MAGYARI, M., KOCH-HENRIKSEN,

N., SORENSEN, P. S., SPELMAN, T., VAN DER WALT, A., HORAKOVA, D., HAVRDOVA, E., GIRARD, M., EICHAU, S., GRAND'MAISON, F., GERLACH, O., TERZI, M., OZAKBAS, S., SKIBINA, O., VAN PESCH, V., SA, M. J., PREVOST, J., ALROUGHANI, R., MCCOMBE, P. A., GOUIDER, R., MRABET, S., CASTILLO-TRIVINO, T., ZHU, C., DE GANS, K., SANCHEZ-MENOYO, J. L., YAMOUT, B., KHOURY, S., SORMANI, M. P., KALINCIK, T., BUTZKUEVEN, H. & BIG, M. S. D. N. 2023. Heterogeneity on long-term disability trajectories in patients with secondary progressive MS: a latent class analysis from Big MS Data network. *J Neurol Neurosurg Psychiatry,* 94**,** 23-30.

SMITH, A. 1982. *Symbol Digit Modalities Test,* Los Angeles, Western Psychological Services.

SNOW, N. J., WADDEN, K. P., CHAVES, A. R. & PLOUGHMAN, M. 2019. Transcranial Magnetic Stimulation as a Potential Biomarker in Multiple Sclerosis: A Systematic Review with Recommendations for Future Research. *Neural Plast,* 2019**,** 6430596.

SOHN, M. N., BROWN, J. C., SHARMA, P., ZIEMANN, U. & MCGIRR, A. 2024. Pharmacological adjuncts and transcranial magnetic stimulation-induced synaptic plasticity: a systematic review. *Journal of Psychiatry & Neuroscience,* 49**,** E59-E76.

SOLEIMANI, G., NITSCHE, M. A., BERGMANN, T. O., TOWHIDKHAH, F., VIOLANTE, I. R., LORENZ, R., KUPLICKI, R., TSUCHIYAGAITO, A., MULYANA, B., MAYELI, A., GHOBADI-AZBARI, P., MOSAYEBI-SAMANI, M., ZILVERSTAND, A., PAULUS, M. P., BIKSON, M. & EKHTIARI, H. 2023. Closing the loop between brain and electrical stimulation: towards precision neuromodulation treatments. *Translational Psychiatry,* 13.

SONDER, J. M., BURGGRAAFF, J., KNOL, D. L., POLMAN, C. H. & UITDEHAAG, B. M. J. 2014. Comparing long-term results of PASAT and SDMT scores in relation to neuropsychological testing in multiple sclerosis. *Multiple Sclerosis Journal,* 20**,** 481-488.

SOROSINA, M., SANTORO, S., FERRE, L., MASCIA, E., CLARELLI, F., GIORDANO, A., CANNIZZARO, M., LUCIA, M., MARTINELLI, V., FILIPPI, M. & ESPOSITO, F. 2023. Risk HLA Variants Affect the T-Cell Repertoire in Multiple Sclerosis. *Neurol Neuroimmunol Neuroinflamm,* 10.

STAMPANONI BASSI, M., GILIO, L., BUTTARI, F., DOLCETTI, E., BRUNO, A., GALIFI, G., AZZOLINI, F., BORRELLI, A., MANDOLESI, G., GENTILE, A., DE VITO, F., MUSELLA, A., SIMONELLI, I., CENTONZE, D. & IEZZI, E. 2024. Preventive exercise and physical rehabilitation promote long-term potentiation-like plasticity expression in patients with multiple sclerosis. *Eur J Neurol,* 31**,** e16071.

STAMPANONI BASSI, M., IEZZI, E. & CENTONZE, D. 2022. Multiple sclerosis: Inflammation, autoimmunity and plasticity. *Handb Clin Neurol,* 184**,** 457-470.

STAMPANONI BASSI, M., IEZZI, E., GILIO, L., CENTONZE, D. & BUTTARI, F. 2019a. Synaptic Plasticity Shapes Brain Connectivity: Implications for Network Topology. *International Journal of Molecular Sciences,* 20.

STAMPANONI BASSI, M., IEZZI, E., MORI, F., SIMONELLI, I., GILIO, L., BUTTARI, F., SICA, F., DE PAOLIS, N., MANDOLESI, G., MUSELLA, A., DE VITO, F., DOLCETTI, E., BRUNO, A., FURLAN, R., FINARDI, A., MARFIA, G. A., CENTONZE, D. & RIZZO, F. R. 2019b. Interleukin-6 Disrupts Synaptic Plasticity and Impairs Tissue Damage Compensation in Multiple Sclerosis. *Neurorehabil Neural Repair,* 33**,** 825-835.

STAMPANONI BASSI, M., IEZZI, E., PAVONE, L., MANDOLESI, G., MUSELLA, A., GENTILE, A., GILIO, L., CENTONZE, D. & BUTTARI, F. 2019c. Modeling Resilience to Damage in Multiple Sclerosis: Plasticity Meets Connectivity. *Int J Mol Sci,* 21.

STAMPANONI BASSI, M., LEOCANI, L., COMI, G., IEZZI, E. & CENTONZE, D. 2018. Can pharmacological manipulation of LTP favor the effects of motor rehabilitation in multiple sclerosis? *Multiple Sclerosis Journal,* 24**,** 902-907.

STAMPANONI BASSI, M., MORI, F., BUTTARI, F., MARFIA, G. A., SANCESARIO, A., CENTONZE, D. & IEZZI, E. 2017. Neurophysiology of synaptic functioning in multiple sclerosis. *Clin Neurophysiol,* 128**,** 1148-1157.

STAMPANONI BASSI, M., NUZZO, T., GILIO, L., MIROBALLO, M., CASAMASSA, A., BUTTARI, F., BELLANTONIO, P., FANTOZZI, R., GALIFI, G., FURLAN, R., FINARDI, A., DE ROSA, A., DI MAIO, A., ERRICO, F., CENTONZE, D. & USIELLO, A. 2021. Cerebrospinal fluid levels of L-glutamate signal central inflammatory neurodegeneration in multiple sclerosis. *Journal of Neurochemistry,* 159**,** 857-866.

STEFAN, K., KUNESCH, E., COHEN, L. G., BENECKE, R. & CLASSEN, J. 2000. Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain,* 123 Pt 3**,** 572-84.

STEFANOU, M. I., DESIDERI, D., BELARDINELLI, P., ZRENNER, C. & ZIEMANN, U. 2018. Phase Synchronicity of μ-Rhythm Determines Efficacy of Interhemispheric Communication Between Human Motor Cortices. *Journal of Neuroscience,* 38**,** 10525-10534.

STEINMAN, L. 2014. Immunology of relapse and remission in multiple sclerosis. *Annu Rev Immunol,* 32**,** 257-81.

STRATTON, S. J. 2021. Population Research: Convenience Sampling Strategies. *Prehospital and Disaster Medicine,* 36**,** 373-374.

STUVE, O., MARRA, C. M., BAR-OR, A., NIINO, M., CRAVENS, P. D., CEPOK, S., FROHMAN, E. M., PHILLIPS, J. T., ARENDT, G., JEROME, K. R., COOK, L., GRAND'MAISON, F., HEMMER, B., MONSON, N. L. & RACKE, M. K. 2006. Altered CD4+/CD8+ T-cell ratios in cerebrospinal fluid of natalizumab-treated patients with multiple sclerosis. *Arch Neurol,* 63**,** 1383-7.

SUCKSDORFF, M., TUISKU, J., MATILAINEN, M., VUORIMAA, A., SMITH, S., KEITILÄ, J., ROKKA, J., PARKKOLA, R., NYLUND, M., RINNE, J., RISSANEN, E. & AIRAS, L. 2019. Natalizumab treatment reduces microglial activation in the white matter of the MS brain. *Neurology-Neuroimmunology & Neuroinflammation,* 6.

SUMOWSKI, J. F. & LEAVITT, V. M. 2013. Cognitive reserve in multiple sclerosis. *Mult Scler,* 19**,** 1122-7.

SUMOWSKI, J. F., WYLIE, G. R., GONNELLA, A., CHIARAVALLOTI, N. & DELUCA, J. 2010. Premorbid cognitive leisure independently contributes to cognitive reserve in multiple sclerosis. *Neurology,* 75**,** 1428-31.

SUPPA, A., QUARTARONE, A., SIEBNER, H., CHEN, R., DI LAZZARO, V., DEL GIUDICE, P., PAULUS, W., ROTHWELL, J. C., ZIEMANN, U. & CLASSEN, J. 2017. The associative brain at work: Evidence from paired associative stimulation studies in humans. *Clin Neurophysiol,* 128**,** 2140-2164.

SUZUKI, M., SAITO, K., MAEDA, Y., CHO, K., ISO, N., OKABE, T., SUZUKI, T. & YAMAMOTO, J. 2023. Effects of Paired Associative Stimulation on Cortical Plasticity in Agonist-Antagonist Muscle Representations. *Brain Sci,* 13.

TAHEDL, M., LEVINE, S. M., GREENLEE, M. W., WEISSERT, R. & SCHWARZBACH, J. V. 2018. Functional Connectivity in Multiple Sclerosis: Recent Findings and Future Directions. *Frontiers in Neurology,* 9.

TAMURA, K., OSADA, T., OGAWA, A., TANAKA, M., SUDA, A., SHIMO, Y., HATTORI, N., KAMAGATA, K., HORI, M., AOKI, S., SHIMIZU, T., ENOMOTO, H., HANAJIMA, R., UGAWA, Y. & KONISHI, S. 2019. MRI-based visualization of rTMS-induced cortical plasticity in the primary motor cortex. *Plos One,* 14.

TAYLOR, R., GALVEZ, V. & LOO, C. 2018. Transcranial magnetic stimulation (TMS) safety: a practical guide for psychiatrists. *Australas Psychiatry,* 26**,** 189-192.
TEDEHOLM, H., LYCKE, J., SKOOG, B., LISOVSKAJA, V., HILLERT, J., DAHLE, C., FAGIUS, J., FREDRIKSON, S., LANDTBLOM, A. M., MALMESTRÖM, C., MARTIN, C., PIEHL, F., RUNMARKER, B., STAWIARZ, L., VRETHEM, M., NERMAN, O. & ANDERSEN, O. 2013. Time to secondary progression in patients with multiple sclerosis who were treated with first generation immunomodulating drugs. *Multiple Sclerosis Journal,* 19**,** 765-774.
TERAO, Y., UGAWA, Y., HANAJIMA, R., MACHII, K., FURUBAYASHI, T., MOCHIZUKI, H., ENOMOTO, H., SHIIO, Y., UESUGI, H., IWATA, N. K. & KANAZAWA, I. 2000. Predominant activation of I1-waves from the leg motor area by transcranial magnetic stimulation. *Brain Res,* 859**,** 137-46.
TERVO, A. E., NIEMINEN, J. O., LIOUMIS, P., METSOMAA, J., SOUZA, V. H., SINISALO, H., STENROOS, M., SARVAS, J. & ILMONIEMI, R. J. 2022. Closed-loop optimization of transcranial magnetic stimulation with electroencephalography feedback. *Brain Stimulation,* 15**,** 523-531.
THALMANN, B., MONSCH, A., BERNASCONI, F., BERRES, M., SCHNEITTER, M., ERMINI-FUENFSCHILLING, D., SPIEGEL, R. & STAEHELIN, H. 1998. Die CERAD Neuropsychologische Testbatterie-Ein gemeinsames minimales Instrumentarium zur Demenzabklärung. *Memory Clinic, Geriatrische Universitätsklinik Basel, Basel*.
THOMPSON, A. J., BANWELL, B. L., BARKHOF, F., CARROLL, W. M., COETZEE, T., COMI, G., CORREALE, J., FAZEKAS, F., FILIPPI, M., FREEDMAN, M. S., FUJIHARA, K., GALETTA, S. L., HARTUNG, H. P., KAPPOS, L., LUBLIN, F. D., MARRIE, R. A., MILLER, A. E., MILLER, D. H., MONTALBAN, X., MOWRY, E. M., SORENSEN, P. S., TINTORÉ, M., TRABOULSEE, A. L., TROJANO, M., UITDEHAAG, B. M. J., VUKUSIC, S., WAUBANT, E., WEINSHENKER, B. G., REINGOLD, S. C. & COHEN, J. A. 2018. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *The Lancet Neurology,* 17**,** 162-173.
TIKSNADI, A., MURAKAMI, T., WIRATMAN, W., MATSUMOTO, H. & UGAWA, Y. 2020. Direct comparison of efficacy of the motor cortical plasticity induction and the interindividual variability between TBS and QPS. *Brain Stimul,* 13**,** 1824-1833.
TOPKA, H. 2007. Motorisch evozierte Potenziale der Armund Handmuskeln. *In:* SIEBNER, H. R. & ZIEMANN, U. (eds.) *Das TMS-Buch: Handbuch der transkraniellen Magnetstimulation.* Berlin, Heidelberg: Springer Berlin Heidelberg.
TREMBLAY, A., CHAREST, K., BRANDO, E., ROGER, E., DUQUETTE, P. & ROULEAU, I. 2023. Cognitive reserve as a moderating factor between EDSS and cognition in multiple sclerosis. *Mult Scler Relat Disord,* 70**,** 104482.
TREMLETT, H., PATY, D. & DEVONSHIRE, V. 2006. Disability progression in multiple sclerosis is slower than previously reported. *Neurology,* 66**,** 172-177.
TRIPEPI, G., JAGER, K. J., DEKKER, F. W. & ZOCCALI, C. 2010. Selection Bias and Information Bias in Clinical Research. *Nephron Clinical Practice,* 115**,** C94-C99.
TROJANO, M., PAOLICELLI, D., BELLACOSA, A. & CATALDO, S. 2003. The transition from relapsing-remitting MS to irreversible disability: clinical evaluation. *Neurol Sci,* 24 Suppl 5**,** S268-70.
TSUTSUMI, R., HANAJIMA, R., TERAO, Y., SHIROTA, Y., OHMINAMI, S., SHIMIZU, T., TANAKA, N. & UGAWA, Y. 2014. Effects of the motor cortical quadripulse transcranial magnetic stimulation (QPS) on the contralateral motor cortex and interhemispheric interactions. *J Neurophysiol,* 111**,** 26-35.
TU, Y. K., BLANCE, A., CLEREHUGH, V. & GILTHORPE, M. S. 2005. Statistical power for analyses of changes in randomized controlled trials. *J Dent Res,* 84**,** 283-7.

TURI, Z., LENZ, M., PAULUS, W., MITTNER, M. & VLACHOS, A. 2021. Selecting stimulation intensity in repetitive transcranial magnetic stimulation studies: A systematic review between 1991 and 2020. *Eur J Neurosci,* 53**,** 3404-3415.
UHER, T., KRASENSKY, J., SOBISEK, L., BLAHOVA DUSANKOVA, J., SEIDL, Z., KUBALA HAVRDOVA, E., SORMANI, M. P., HORAKOVA, D., KALINCIK, T. & VANECKOVA, M. 2018. Cognitive clinico-radiological paradox in early stages of multiple sclerosis. *Ann Clin Transl Neurol,* 5**,** 81-91.
UYGUR-KUCUKSEYMEN, E., PACHECO-BARRIOS, K., YUKSEL, B., GONZALEZ-MEGO, P., SOYSAL, A. & FREGNI, F. 2023. Non-invasive brain stimulation on clinical symptoms in multiple sclerosis patients: A systematic review and meta-analysis. *Mult Scler Relat Disord,* 78**,** 104927.
VALLENCE, A. M., RURAK, B. K., FUJIYAMA, H. & HAMMOND, G. R. 2023. Covariation of the amplitude and latency of motor evoked potentials elicited by transcranial magnetic stimulation in a resting hand muscle. *Exp Brain Res,* 241**,** 927-936.
VAN DEN BOS, M. A., GEEVASINGA, N., MENON, P., BURKE, D., KIERNAN, M. C. & VUCIC, S. 2017. Physiological processes influencing motor-evoked potential duration with voluntary contraction. *J Neurophysiol,* 117**,** 1156-1162.
VAN DER WEELE, T. J. 2019. Principles of confounder selection. *European Journal of Epidemiology,* 34**,** Cp3-219.
VAN GINKEL, J. R. & KROONENBERG, P. M. 2021. Multiple Imputation to Balance Unbalanced Designs for Two-Way Analysis of Variance. *Methodology-European Journal of Research Methods for the Behavioral and Social Sciences,* 17**,** 39-57.
VAN NIEROP, G. P., VAN LUIJN, M. M., MICHELS, S. S., MELIEF, M. J., JANSSEN, M., LANGERAK, A. W., OUWENDIJK, W. J. D., HINTZEN, R. Q. & VERJANS, G. 2017. Phenotypic and functional characterization of T cells in white matter lesions of multiple sclerosis patients. *Acta Neuropathol,* 134**,** 383-401.
VAVASOUR, I. M., SUN, P., GRAF, C., YIK, J. T., KOLIND, S. H., LI, D. K., TAM, R., SAYAO, A. L., SCHABAS, A., DEVONSHIRE, V., CARRUTHERS, R., TRABOULSEE, A., MOORE, G. W., SONG, S. K. & LAULE, C. 2022. Characterization of multiple sclerosis neuroinflammation and neurodegeneration with relaxation and diffusion basis spectrum imaging. *Mult Scler,* 28**,** 418-428.
VIGNE, M., KWEON, J., SHARMA, P., GREENBERG, B. D., CARPENTER, L. L. & BROWN, J. C. 2023. Chronic caffeine consumption curbs rTMS-induced plasticity. *Frontiers in Psychiatry,* 14.
VIGNOLI, B., BATTISTINI, G., MELANI, R., BLUM, R., SANTI, S., BERARDI, N. & CANOSSA, M. 2016. Peri-Synaptic Glia Recycles Brain-Derived Neurotrophic Factor for LTP Stabilization and Memory Retention. *Neuron,* 92**,** 873-887.
VOIGT, J., CARPENTER, L. & LEUCHTER, A. 2017. Cost effectiveness analysis comparing repetitive transcranial magnetic stimulation to antidepressant medications after a first treatment failure for major depressive disorder in newly diagnosed patients - A lifetime analysis. *PLoS One,* 12**,** e0186950.
WAHL, M., HUEBERS, A., LAUTERBACH-SOON, B., HATTINGEN, E., JUNG, P., COHEN, L. G. & ZIEMANN, U. 2011. Motor Callosal Disconnection in Early Relapsing-Remitting Multiple Sclerosis. *Human Brain Mapping,* 32**,** 846-855.
WALTON, C., KING, R., RECHTMAN, L., KAYE, W., LERAY, E., MARRIE, R. A., ROBERTSON, N., LA ROCCA, N., UITDEHAAG, B., VAN DER MEI, I., WALLIN, M., HELME, A., ANGOOD NAPIER, C., RIJKE, N. & BANEKE, P. 2020. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. *Mult Scler,* 26**,** 1816-1821.
WANG, Y. A., SPARKS, J., GONZALES, J. E., HESS, Y. D. & LEDGERWOOD, A. 2017. Using independent covariates in experimental designs: Quantifying the trade-off

between power boost and Type I error inflation. *Journal of Experimental Social Psychology,* 72**,** 118-124.
WANKERL, K., WEISE, D., GENTNER, R., RUMPF, J. J. & CLASSEN, J. 2010. L-type voltage-gated Ca2+ channels: a single molecular switch for long-term potentiation/long-term depression-like plasticity and activity-dependent metaplasticity in humans. *J Neurosci,* 30**,** 6197-204.
WASSERMANN, E. M. 1998. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. *Electroencephalogr Clin Neurophysiol,* 108**,** 1-16.
WEGNER, C., ESIRI, M. M., CHANCE, S. A., PALACE, J. & MATTHEWS, P. M. 2006. Neocortical neuronal, synaptic, and glial loss in multiple sclerosis. *Neurology,* 67**,** 960-7.
WEINER, H. L. 2004. Multiple sclerosis is an inflammatory T-cell-mediated autoimmune disease. *Arch Neurol,* 61**,** 1613-5.
WEISE, D., MANN, J., RIDDING, M., ESKANDAR, K., HUSS, M., RUMPF, J. J., DI LAZZARO, V., MAZZONE, P., RANIERI, F. & CLASSEN, J. 2013. Microcircuit mechanisms involved in paired associative stimulation-induced depression of corticospinal excitability. *J Physiol,* 591**,** 4903-20.
WIRSCHING, I., BUTTMANN, M., ODORFER, T., VOLKMANN, J., CLASSEN, J. & ZELLER, D. 2018. Altered motor plasticity in an acute relapse of multiple sclerosis. *Eur J Neurosci,* 47**,** 251-257.
WITTKOPF, P. G., LARSEN, D. B. & GRAVEN-NIELSEN, T. 2021. Protocols for inducing homeostatic plasticity reflected in the corticospinal excitability in healthy human participants: A systematic review and meta-analysis. *Eur J Neurosci,* 54**,** 5444-5461.
ZELLER, D., AUFM KAMPE, K., BILLER, A., STEFAN, K., GENTNER, R., SCHUTZ, A., BARTSCH, A., BENDSZUS, M., TOYKA, K. V., RIECKMANN, P. & CLASSEN, J. 2010. Rapid-onset central motor plasticity in multiple sclerosis. *Neurology,* 74**,** 728-35.
ZELLER, D. & CLASSEN, J. 2014. Plasticity of the motor system in multiple sclerosis. *Neuroscience,* 283**,** 222-30.
ZIEMANN, U. & SIEBNER, H. R. 2015. Inter-subject and Inter-session Variability of Plasticity Induction by Non-invasive Brain Stimulation: Boon or Bane? *Brain Stimulation,* 8**,** 662-663.
ZIERFUSS, B., LAROCHELLE, C. & PRAT, A. 2024. Blood-brain barrier dysfunction in multiple sclerosis: causes, consequences, and potential effects of therapies. *Lancet Neurol,* 23**,** 95-109.
ZIGMOND, A. S. & SNAITH, R. P. 1983. The hospital anxiety and depression scale. *Acta Psychiatr Scand,* 67**,** 361-70.
ZORUMSKI, C. F. & IZUMI, Y. 2012. NMDA receptors and metaplasticity: Mechanisms and possible roles in neuropsychiatric disorders. *Neuroscience and Biobehavioral Reviews,* 36**,** 989-1000.
ZRENNER, C., DESIDERI, D., BELARDINELLI, P. & ZIEMANN, U. 2018. Real-time EEG-defined excitability states determine efficacy of TMS-induced plasticity in human motor cortex. *Brain Stimul,* 11**,** 374-389.
ZRENNER, C. & ZIEMANN, U. 2024. Closed-Loop Brain Stimulation. *Biological Psychiatry,* 95**,** 545-552.

Acknowledgements

I would like to thank my promoters Prof. Dr. med. Philipp Albrecht, Dr. med. Stefan Jun Groiss and PD Dr. med Thomas Schroeder for their supervision of my thesis project. Moreover, I would like to thank Prof. Dr. med. Philipp Albrecht and Dr. med. Stefan Jun Groiss, who let me be a part of their research project on which my thesis was based and for providing me with this interesting research question.

I also like to thank Dr. rer. nat. Carolin Balloff for her role in coordinating the research project.

Great thanks to my sister PhD Jana Stucke, for proofreading and building up my spirit at times when the writing process was hard.

My special thanks go out to my mother Dr. med Livia Stucke and my father Friedhelm Stucke, who always supported my goal of pursuing a career in medicine. Without you, this thesis would not have been possible.