Quadripulse-stimulation-induced plasticity in patients with multiple sclerosis and its functional relevance

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List of abbreviations
(articles excluded)

BCM ..... Bienenstock-Cooper-Munro rule
CIS ..... clinically isolated syndrome
CNS ..... central nervous system
COVID-19 ..... coronavirus disease 2019
CSP ..... cortical silent period
EDSS ..... Expanded Disability Status Scale
EEG ..... electroencephalography
EMG ..... electromyography
IPS ..... information processing speed
LMEM ..... linear-mixed-effects models
LTD ..... long-term depression
LTP ..... long-term potentiation
M1 ..... primary motor cortex
MEP ..... motor-evoked potential
MRI ..... magnetic resonance imaging
MS ..... multiple sclerosis
PAS 25 ..... paired associative simulation protocol with an interstimulus interval of 25ms
PIRA ..... progression independent of relapse activity
PMS ..... primary or secondary progressive multiple sclerosis
PPMS ..... primary progressive multiple sclerosis
QPS ..... quadripulse stimuation
QPS-5 ..... quadripulse stimulation with an inter-stimulus interval of 5ms
RIS ..... radiologically isolated syndrome
ROC ..... receiver-operating characteristic
RRMS ..... relapsing-remitting multiple sclerosis
rTMS ..... repetitive transcranial magnetic stimulation
SDMT ..... Symbol Digit Modalities Test
SPMS ..... secondary progressive multiple sclerosis
TBS ..... theta burst stimulation
TMS ..... transcranial magnetic stimulation

Abstract

Despite advancements in understanding the pathophysiology of multiple sclerosis (MS), predicting individual clinical trajectories remains elusive. Compensatory mechanisms of neuroplasticity are gaining recognition as potentially significant contributors to shaping clinical outcomes and may hold prognostic value for disease progression. Synaptic plasticity, an early-phase neuroplasticity mechanism, can be non-invasively investigated at the motor cortex using repetitive transcranial magnetic stimulation (rTMS). In patients with MS, the quadripulse-stimulation (QPS) protocol in particular shows promise for effective induction of synaptic plasticity.

This thesis investigated QPS-induced plasticity in patients with MS both in cross-sectional and longitudinal contexts. Four empirical studies were conducted to compare plasticity across MS subtypes and healthy controls (HCs), assess its correlation with cognitive and motor function, study alterations in plasticity during acute relapses, and analyze its association with disease progression over time. The primary aim was to investigate QPS-induced plasticity as a potential biomarker for predicting disease progression.

The first study revealed a positive correlation between cognitive performance and QPS-induced plasticity in patients with relapsing-remitting MS (RRMS), with plasticity serving as a distinguishing factor between patients with and without cognitive impairment. RRMS patients did not exhibit diminished plasticity compared to HCs. In the second study, QPS-induced plasticity did not significantly differ between patients with MS during acute relapses, stable patients with MS, and HCs. Exploratory findings suggested higher plasticity in relapsing patients with motor disability. Similarly, the third study found no significant differences in QPS-induced plasticity among patients with different MS subtypes and HCs. Additionally, correlations with motor and cognitive functions were evident only in MS patients with intact corticospinal tract integrity. Longitudinal analysis in the fourth study revealed that patients experiencing clinically relevant decline in manual dexterity or visuospatial short-term learning and memory after a median follow-up of two years exhibited lower levels of baseline synaptic plasticity. However, overall functional outcomes remained relatively stable over time, with a similar number of patients experiencing improvement and decline.

In summary, this thesis indicates preserved QPS-induced plasticity across all MS subtypes and disease activity levels. Furthermore, it highlights the need to consider clinical characteristics in synaptic plasticity research in patients with MS and proposes a potential link between the degree of QPS-induced plasticity and functional decline. However, the role of QPS-induced plasticity as an independent biomarker for predicting disease progression at the individual level currently remains uncertain due to various methodological challenges.

1. Introduction

Multiple Sclerosis (MS) is an autoimmune inflammatory, demyelinating, and degenerative chronic disease of the central nervous system *(*CNS*).* Lesions can occur anywhere in the CNS, affecting both gray and white matter (Baecher-Allan et al., 2018; Di Filippo et al., 2018). Consequently, a variety of symptoms can emerge ranging from 'visible' physical symptoms, such as bladder dysfunction and spasticity, to 'hidden' symptoms, such as cognitive impairment and fatigue (Compston & Coles, 2008; Katz Sand, 2015; Lysandropoulos & Havrdova, 2015).

Epidemiological data suggest a global prevalence rate of 36 per 100,000 people, meaning that approximately 2.8 million people are currently affected by MS worldwide (Walton et al., 2020). Although prevalence rates have risen within the last centuries in every world region, there's great variation across the globe. With a rate of 303 per 100,000 Germany has the second highest prevalence worldwide (The Multiple Sclerosis International Federation [MSIF], 2020). Independent of the world region, there is a twofold increased risk for females compared to males (defined as sex assigned at birth) and the average age of diagnosis is 32 years (Walton et al., 2020). Due to the early onset of the disease within the life span, MS is the most common neurological disease leading to disability in young adults (MSIF, 2020) and places a large personal as well as socioeconomic burden (Bebo et al., 2022; Mitchell et al., 2005; Paz-Zulueta et al., 2020).

Importantly, correlations between lesion load and clinical symptoms are generally poor (Barkhof, 2002). Despite the presence of significant radiological abnormalities, some patients may experience no or only mild symptoms, whereas others may experience severe symptoms despite minimal radiological findings. This discrepancy between clinical symptoms and radiological findings is referred to as the 'clinico-radiological paradox' (Barkhof, 2002) and suggests that disease progression and disability are determined by multiple factors.

In addition to remyelination (Albert et al., 2007) and lesion location (strongly connected region, i.e. hub, or area of functional redundancy (Schoonheim et al., 2022)), compensatory mechanisms of neuroplasticity may play an important role (Zeller & Classen, 2014). Neuroplasticity may not only contribute to recovery of clinical symptoms but may also prevent them in the first place. If the structural and functional damage exceeds the compensatory reserve, CNS injury may manifest in clinical symptoms (Zeller & Classen, 2014). Therefore, it is of great interest to reliably assess and quantify neuroplasticity and to evaluate its prognostic value for the clinical course in patients with MS.

Different methods have emerged to assess cortical plasticity, i.e. neuroplasticity at the cortical level, in both healthy and diseased brains. In addition to behavioral assessments and functional brain imaging, repetitive transcranial magnetic stimulation (rTMS) of the motor cortex has been introduced as a promising non-invasive technique (Barker et al., 1985). It can

modulate synaptic efficacy by strengthening or weakening existing synapses, which is referred to as long-term potentiation (LTP) and long-term depression (LTD), respectively (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973; Dudek & Bear, 1992). The degree of modulation induced by rTMS serves as a proxy for rapid-onset cortical plasticity (Zeller & Classen, 2014).

The following chapters will introduce relevant pathophysiological and clinical aspects of MS. Moreover, detailed information on neuroplasticity, as well as the applied method, namely rTMS, and the quadripulse-stimulation (QPS) protocol in particular, will be provided. Subsequently, the current state of research on rTMS-induced plasticity in patients with MS will be reviewed, leading to the aim of this thesis. Four original research articles addressing specific objectives and hypotheses will then be presented. Finally, the results will be summarized and discussed.

1.1. Pathophysiology of MS and phenotypes

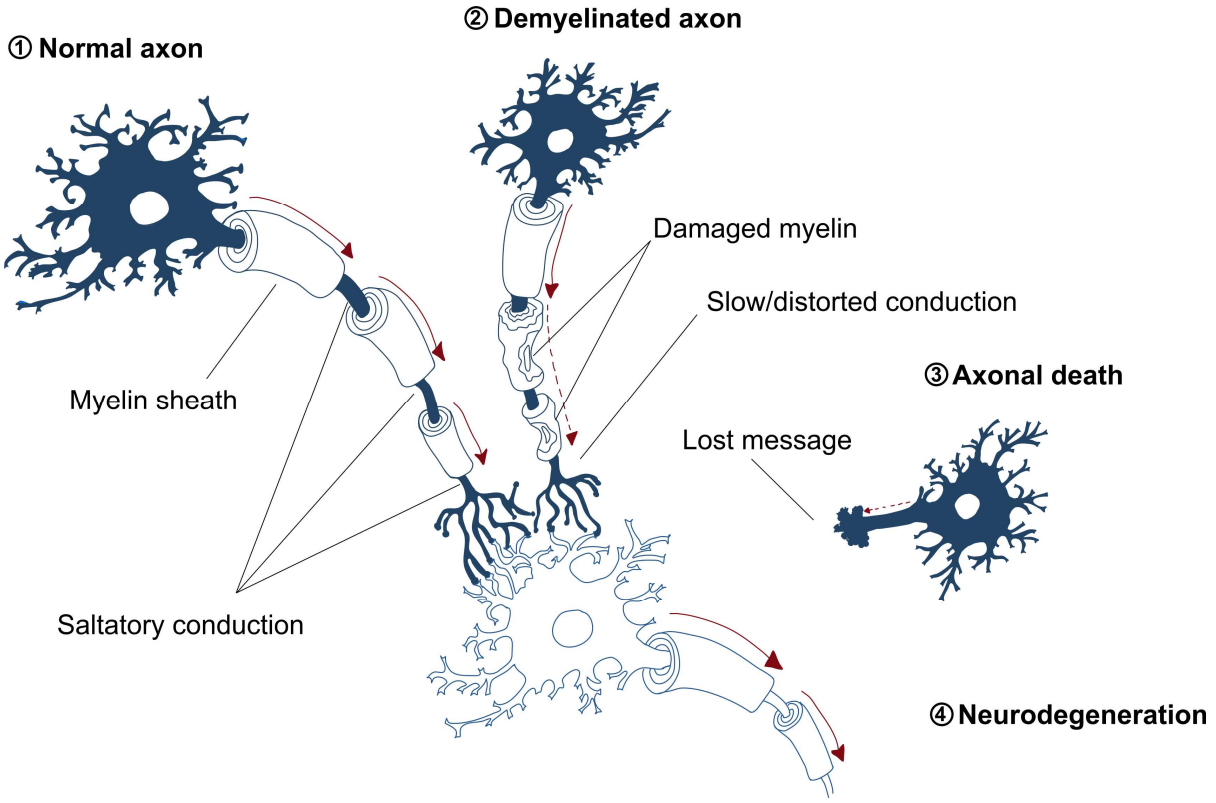
MS is characterized by 1) inflammatory lesions, primarily affecting myelin sheaths and oligodendrocytes (Lassmann, 2018), and 2) neurodegeneration (Sandi et al., 2021). While it remains unclear which of these processes is the primary initiator of MS pathology, both appear to be present at disease onset (Sandi et al., 2021).

Oligodendrocytes do not only myelinate axons but also serve other important functions supporting axonal health (Simkins et al., 2021). Myelin is critical to increase the speed of action potential propagation and thus signal conduction between neurons (Hartline & Colman, 2007). If myelin and oligodendrocytes are destroyed, nerve conduction velocity is reduced or entirely lost (Koles & Rasminsky, 1972) and the loss of their protective functions can lead to axonal damage, transection, and loss (Kornek et al., 2000; Lovas et al., 2000; Trapp et al., 1998).

After acute inflammatory demyelination, lesions can remain chronically active with detrimental clinical long-term effects (Absinta et al., 2019). Remyelination of demyelinated axons is possible, but highly variable across patients and lesion location, typically failing in periventricular lesions (Albert et al., 2007; Goldschmidt et al., 2009; Patrikios et al., 2006; Tonietto et al., 2023). The periventricular failure of remyelination may contribute to neurodegeneration (Tonietto et al., 2023), which also occurs diffusely in normal-appearing white and gray matter (Lassmann, 2018) and is already present early in the disease process (Hauser & Oksenberg, 2006). In addition to direct inflammatory damage to myelin, oligodendrocytes, and axons, indirect effects such as Wallerian degeneration (Dziedzic et al., 2010), mitochondrial dysfunction (Witte et al., 2014), and oxidative burst activation of microglia and macrophages (Fischer et al., 2012) contribute to neurodegeneration. Consequently, brain atrophy rates are higher in patients with MS compared to the general population (Stefano et al., 2016). Demyelination and axonal injury typically dominate early in the disease, while enlargement of lesions in normal-appearing white and gray matter and neurodegeneration are

more pronounced in the progressive phase (Sandi et al., 2021). *Figure 1* summarizes and illustrates the most important pathophysiological mechanisms of MS.

Figure 1. *Pathophysiological mechanisms of MS*.



Note. In the healthy brain, axons are surrounded by myelin. Interruptions in the myelin sheath along a myelinated axon are called Ranvier's node. The signal propagates only from one Ranvier's node to the next, facilitating rapid conduction known as 'saltatory conduction' (part 1). If the myelin is damaged, signal propagation becomes slower or distorted (part 2). Additionally, the axon is vulnerable to attacks and degenerates (part 3), which ultimately results in degeneration of the entire nerve cell (part 4). Adapted from The National Multiple Sclerosis Society (2024a).

Based on the rate of progression, MS is currently classified into four clinical courses. Patients with a first MS-typical clinical presentation can be diagnosed with clinically isolated syndrome (CIS), if the symptoms are suggestive of inflammatory demyelination but the full diagnostic criteria of MS are not yet fulfilled (please refer to appendix A for the detailed diagnostic criteria) (Lublin et al., 2014). Despite high variability of conversion rates across studies, most patients diagnosed with CIS are later diagnosed with clinically definite MS (Marcus & Waubant, 2013). For most patients (~85%), the disease initially presents as relapsing-remitting MS (RRMS). RRMS is characterized by episodes of sudden evolution or exacerbation of neurological symptoms on the one hand, and episodes of remission of these

symptoms and clinical stability on the other (Klineova & Lublin, 2018). As the disease progresses over time, remission remains incomplete more often, and most patients (>80%) with untreated RRMS develop secondary progressive MS (SPMS) within 25 years (Scalfari et al., 2010). However, disease modifying therapies can reduce the conversion rate markedly (Cree et al., 2016; Lublin et al., 2022). In SPMS, symptoms worsen progressively with or without acute exacerbations (Lublin et al., 2014). Due to this insidious accumulation of disability, diagnosis of SPMS is challenging and typically made retrospectively (Klineova & Lublin, 2018). Importantly, recent evidence suggests an important role of progression independent of relapse activity (PIRA) in patients with RRMS as well (Cagol et al., 2022; Kappos et al., 2020; Lublin et al., 2022). Unlike patients with RRMS and SPMS, those with primary progressive MS (PPMS) do not experience acute exacerbations, but instead face a gradual worsening of symptoms from the disease's onset (Lublin & Reingold, 1996). Approximately 10-20% of patients with MS suffer from this disease type (Miller & Leary, 2007).

Radiologically isolated syndrome (RIS) affects individuals who present no clinical signs but brain imaging findings indicating inflammatory demyelination. Due to the lack of specificity of these imaging findings, RIS is currently not classified as an MS subtype (Lublin et al., 2014). However, 34% of these patients experience a first acute or progressive clinical event within five years (Okuda 2014). Therefore, close monitoring of individuals with RIS is warranted (Lublin et al., 2014).

MS lesions can potentially occur anywhere in the CNS, but are typically located juxtacortical, cortical, periventricular and infratentorial, as well as in the corpus callosum and the cervical segment of the spinal cord (Filippi et al., 2019). Although myelin concentration is higher in white compared to gray matter (Corrigan et al., 2021), lesions occur in both types of brain tissue (Hulst & Geurts, 2011). Thus, various clinical symptoms can arise, which will be outlined in the next chapter.

1.2. Clinical symptoms and disease progression

MS can manifest with a wide range of clinical symptoms and severity. However, bowel/bladder, mobility and sexual dysfunction, visual disturbances, spasticity, sensory disturbances, pain, dizziness, cognitive impairment, fatigue, and depression are the most typical symptoms (The National Multiple Sclerosis Society, 2024b). Within the first year of the disease, sensory disturbances and fatigue are the most frequently reported symptoms, affecting 85% and 81% of patients, respectively. Even at this early stage of the disease, 15% require mobility assistance at least occasionally and 63% report subjective change in their cognitive functions (Kister et al., 2013). Consequently, patients with MS often experience a reduced quality of life (Benedict et al., 2005), and leave the workforce (Kobelt et al., 2017; Langdon, 2011; Renner et al., 2020).

Cognitive impairment occurs with a prevalence of 34-65% and typically includes deficits in information processing speed (IPS), learning, memory, executive functioning, and visuospatial processing. Despite high variability across patients, IPS, learning and memory are most frequently and earliest impaired (Benedict et al., 2020; Wojcik et al., 2022). Recent evidence suggests that IPS depends on the intact functioning of extensive networks rather than single structures. Thus, focal lesions compromising these complex connections can lead to impaired IPS early in the disease course (Macaron et al., 2020; Tóth et al., 2019). Learning and memory functions, however, may be associated with spreading pathology in the gray matter as well as brain atrophy, especially in the hippocampus (Tóth et al., 2019).

In addition to their genuine, onerous effects, cognitive deficits can also have detrimental secondary effects, e.g. poor treatment adherence (Roy et al., 2016). In general, cognitive impairment is accentuated in patients with progressive types of MS (PMS) compared to patients with RRMS (Benedict et al., 2020; Johnen et al., 2017; Sonneville et al., 2002). Cognitive performance declines with disease progression independently of normal ageing effects (Amato et al., 2006) and, with time, deficits arise in previously unaffected domains (Achiron et al., 2013).

Disease progression is highly variable across subjects and is either acquired relapse-associated or through PIRA (Lublin et al., 2022). Global brain tissue loss in patients with PIRA and those with relapse activity appears to be similar (Cagol et al., 2022; Cree et al., 2019). However, the specific mechanisms underlying disability accumulation are not yet fully understood (Cagol et al., 2022). Generally, male sex is associated with faster disability accumulation (Alvarez-Sanchez 2022). Further, older age and higher levels of pre-existing disability are linked to incomplete recovery from relapses, thereby contributing to disability accumulation (Lublin et al., 2022). Several potential biomarkers of disease activity and progression have been investigated, but despite their prognostic value on the group level, their clinical use on the individual level is limited (Yang et al., 2022). For example, significant disparities between lesion load observed in brain imaging and clinical symptoms have been identified and termed the ‘clinico-radiological paradox’ (Barkhof, 2002) which will be further elucidated in the subsequent chapter.

1.3. Clinico-radiological paradox

The ‘clinico-radiological paradox' describes the inconsistent correlation between clinical symptoms experienced by patients with MS and the extent or severity of radiological findings. While some patients exhibit significant clinical disability despite minimal or non-visible lesions on magnetic resonance imaging (MRI) scans, others have few clinical symptoms despite extensive lesions (Barkhof, 2002). In line with this, cognitive performance was found

to be only weakly to moderately correlated with T2 MRI lesion burden in a large meta-analysis (Mollison et al., 2017).

Since Barkhof's (2002) initial description of this phenomenon, significant advances have been made to better understand the relationship between brain lesions and clinical symptoms. These advances include insights into the relevance of lesion location, as well as structural and functional brain network connectivity (Carotenuto, Valsasina, et al., 2022; Cipriano et al., 2023; Eshaghi et al., 2018; Hackmack et al., 2012; Lapucci et al., 2022; Pardini et al., 2015; Soares et al., 2021; Welton et al., 2020; Zivadinov et al., 2016). Additionally, associations between symptoms and lesions appear to vary depending on the disease stage, with weaker correlations in patients with short disease durations (Uher et al., 2018). Although no significant longitudinal differences in atrophy rates have been detected between RRMS and PMS patients (Kalkers et al., 2002; Stefano et al., 2010; Tsagkas et al., 2020), recent research has revealed an association between brain atrophy, particularly gray matter loss in frontal and parietal areas, and PIRA in RRMS patients (Cagol et al., 2022).

Despite these advances in overcoming the 'clinico-radiological paradox', several confounding factors can influence the longitudinal assessment of brain volume and lesion load. These include individual-level variables, such as age, sex, brain size, hydration state, physical activity, alcohol consumption, cigarette smoking, substance abuse, and somatic comorbidities, as well as technical factors like variations in the quality of the MRI scanner and analysis software (Sastre-Garriga et al., 2020).

Thus, the clinical utility of specialized MRI measures at the individual level is limited. However, the 'clinico-radiological paradox' may not only be explained by more advanced imaging and analysis techniques, but also by other factors, such as compensatory neuroplasticity, which will be described in detail in the next chapter.

1.4. Neuroplasticity and cortical plasticity

Neuroplasticity is a dynamic process of the CNS to adapt to alterations in the internal or external environment (Buonomano & Merzenich, 1998; Sharma et al, 2013). It is, thus, a core feature of neuronal function underlying learning (Dimyan & Cohen, 2011; Scholz et al., 2009), and memory (Martin et al., 2000). It is, however, not only important in the healthy but also in the lesioned brain, as numerous studies have demonstrated adaptation of the CNS after brain injury in primates (Dijkhuizen et al., 2001; Dijkhuizen et al., 2003; Nudo & Milliken, 1996; Wei et al., 2001) and humans (Calautti et al., 2001; Centonze et al., 2007; Cicinelli et al., 1997; Takeda et al., 2007; Traversa et al., 1997; Turton et al., 1996). In fact, recovery following brain injury primarily relies on the restoration of functional and structural connectivity (Stampanoni Bassi et al., 2017).

Functional plasticity refers to the reorganization or restoration of activity of existing neural circuits in response to input patterns (Magee & Grienberger, 2020). It involves changes in neuronal membrane excitability (Clarkson et al., 2010) or the strength of (single) synapses (Hebb, 1949), as well as recruitment of inactive synapses (Jacobs & Donoghue, 1991).

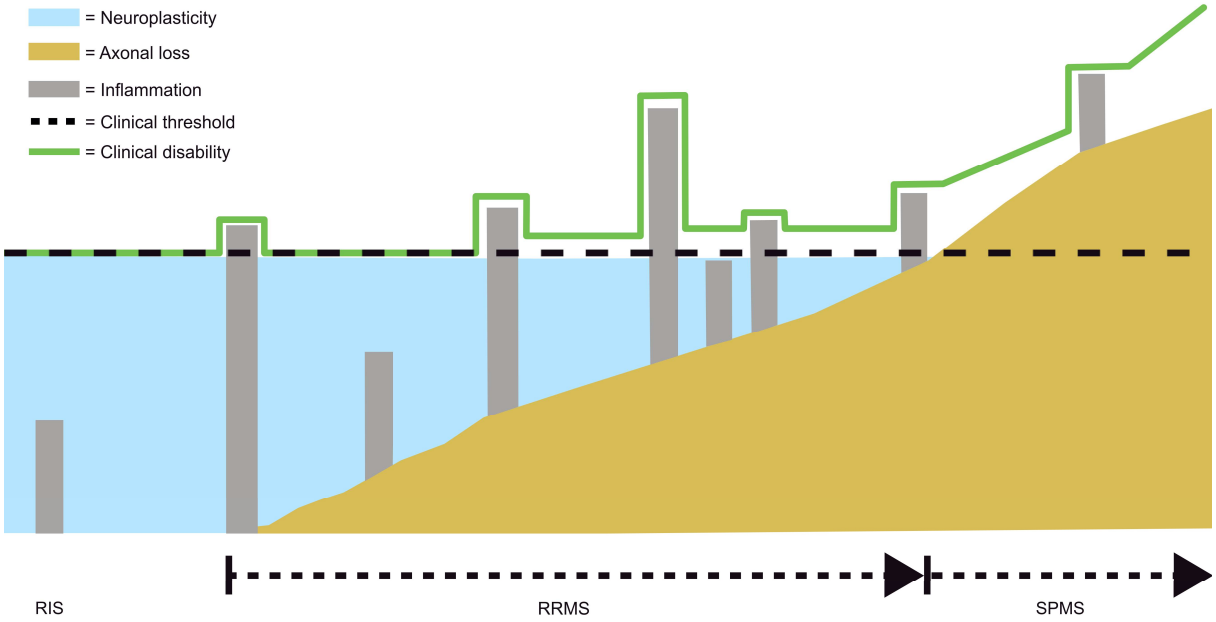
Structural plasticity involves physical changes, e.g. axonal sprouting (Carmichael et al., 2017), changes in sodium channel density (Cantrell & Catterall, 2001), dendritic and axonal anatomy (Jamann et al., 2018; Kasai et al., 2010), remyelination (Patrikios et al., 2006), synaptogenesis (Andersen & Soleng, 1998) and neurogenesis (Eriksson et al., 1998).

While functional changes can occur rapidly within minutes, structural alterations take months to years (Zeller & Classen, 2014). Importantly, rapid functional and late-onset structural changes do not occur independently of each other. It has been suggested that rapid-onset electrophysiological events can finally induce biochemical and morphological events, which evolve more slowly but are more persistent (Classen et al., 1998; Ugawa, 2012). Thus, both types of plasticity collectively guarantee the adaptability of the CNS.

Neuroplasticity has been incorporated into different models of MS disease progression (Krieger et al., 2016; Schoonheim et al., 2022; Schoonheim et al., 2010). Taken together, these models suggest that neuroplasticity serves as a protective factor against clinical disability arising from structural damage. According to these models, disability accumulation occurs when structural damage exceeds the compensatory capacity of neuroplasticity, which is limited and varies among individuals. As neuroplasticity can (temporarily) ‘silence’ the clinical consequences of lesions, it may help to understand the ‘clinico-radiological paradox’ in MS, which is crucial for optimizing patient management and treatment decisions.

Figure 2 illustrates the key aspects of neuroplasticity in the context of the pathophysiological mechanisms in patients with MS.

Figure 2. *Neuroplasticity in relation to the pathophysiological mechanisms of MS.*



Note. Neuroplasticity can be compared to a backup reservoir capable of preventing the effects of inflammation and axonal loss from surpassing a theoretical clinical threshold. Once this threshold is breached, symptoms manifest. Neuroplasticity wanes as inflammation and axonal loss intensify, and if it's depleted, further brain tissue loss and inflammation directly translate into clinical disability. Patients with RRMS experience periods of clinical stability, whereas those with SPMS experience a progressive increase in disability over time. Adapted from Krieger et al. (2016), Schoonheim et al. (2010), and Compston and Coles (2002).

Whereas neuroplasticity broadly refers to the brain's overall capacity to change and adapt, cortical plasticity specifically refers to adaptive changes in the cerebral cortex, i.e., the outermost layer of the cerebrum. It is, thus, a subset of neuroplasticity, and has been the target of most studies of neuroplasticity in humans due to its exposed and thus accessible location (Groppa et al., 2012; Jannati et al., 2023) (more details on the assessment of cortical plasticity are provided in chapter *1.5.2. Evaluating synaptic plasticity using rTMS*). One of the early-phase events of neuroplasticity is synaptic plasticity (Matsumoto & Ugawa, 2020), which will be explained in more detail in the following section.

1.5. Synaptic plasticity

Synaptic plasticity refers to activity-dependent alterations in the strength of synaptic connections (Magee & Grienberger, 2020), i.e. the effectiveness of the influence of the presynaptic on the postsynaptic neuron (Murthy, 1998). Although more complex mechanisms seem to be involved as well (Magee & Grienberger, 2020), the basic principles of activity-dependent synaptic changes have been described in Hebb's learning rules. According to Hebb (1949), the synaptic connection between two neurons is strengthened if one neuron consistently contributes to the activity of the other. By linking input and output patterns, the

output pattern may even be evoked if only fragments of the input pattern are present (Magee & Grienberger, 2020). First described in the hippocampus of rabbits (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973), an increase in the synaptic strength after repetitive high-frequency stimulation became known as LTP. Its counterpart, LTD, was revealed by Dudek and Bear (1992) by testing the theoretical prediction of the Bienenstock-Cooper-Munro rule (BCM) (Bienenstock et al., 1982). According to the BCM, each neuron possesses a critical synaptic modification threshold, determining whether LTP or LTD is induced. If excitatory synaptic inputs consistently yield postsynaptic responses greater than the modification threshold, these inputs are potentiated. If they consistently yield postsynaptic responses below the threshold, thus failing to activate the postsynaptic neuron, they are depressed (Bienenstock et al., 1982). Indeed, Dudek and Bear (1992) showed that low-frequency presynaptic stimulation depressed postsynaptic activity.

1.5.1. Metaplasticity

As described above, the modification threshold determines whether activation of an excitatory input leads to LTP or LTD. Importantly, the threshold is dynamically adjusted based on the average postsynaptic activity. The higher the prolonged preceding activity, the higher the threshold, preventing further LTP but facilitating LTD. In turn, low levels of postsynaptic activity decrease the threshold, thus increasing the chances for LTP and preventing further LTD (Bienenstock et al., 1982). These changes in the ability to undergo subsequent LTP and LTD have been termed metaplasticity and may protect against excitotoxicity (Abraham & Bear, 1996). The fundamental components of metaplasticity are postulated to encompass changes in synaptic strength and neurotransmitter release (Turrigiano & Nelson, 2004), neuromodulatory systems (Meunier et al., 2017), signaling pathways (Kelleher et al., 2004) and the activity of microglia and immune molecules (Wu et al., 2015). However, our understanding of the precise mechanisms governing metaplasticity remains limited (Cantone et al., 2021), with a detailed exploration falling beyond the scope of this thesis.

1.5.2. Evaluating synaptic plasticity using rTMS

LTP and LTD can not only be induced endogenously through repeated experiences and exercises, but also through external stimulation. RTMS is one of the most utilized non-invasive brain stimulation techniques to modulate cortical excitability by inducing effects similar to LTP and LTD (Antal et al., 2022). It is based on transcranial magnetic stimulation (TMS), which was introduced by Barker et al. (1985) as a pain-free alternative to transcranial electrical stimulation of the human motor cortex. While transcranial electrical stimulation directly induces an electrical current to the scalp using electrodes, TMS is based on electromagnetic induction (Barker et al., 1985).

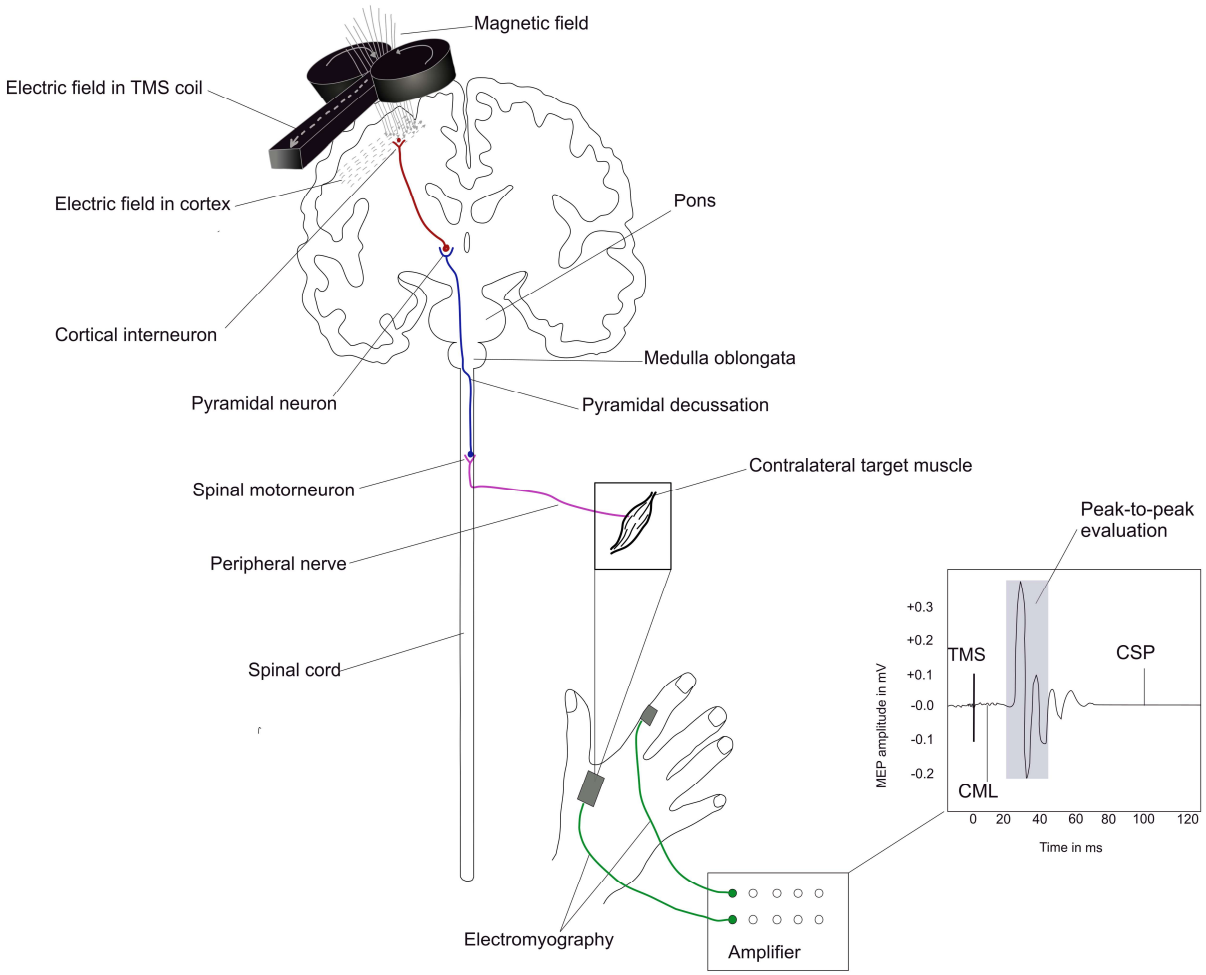
A transducing coil is placed tangentially to the scalp and attached to a high-voltage (400 V–3 kV), high-current (4 kA–20 kA) discharge system (Jalinous, 1991). A discharge of the system induces an electric current within the coil, which induces a strong but short-lasting magnetic field on the scalp (1-2.5 tesla for ≤1 ms). The magnetic field penetrates the skull and dura and generates an electrical field within the cortex (Groppa et al., 2012; Neva et al., 2020). The resultant current flow can activate neurons by causing action potentials (Siebner et al., 2022). However, the direct neural response to the induced electric field is complex, involving a cascade of high-frequency synaptic activity, depending on numerous factors, e.g. the orientation of the axon relative to the electric field, the magnitude of the electric field, the stimulation intensity, and the pulse waveform (mono- or biphasic) (Siebner et al., 2022). For both waveforms, the induced current flow in one direction (positive or negative) is always counterbalanced by an equal flow of current in the opposite direction (Groppa et al., 2012). In monophasic TMS, the natural oscillation of the current flow between capacitor and coil is dampened (Wendt et al., 2023). Only the initial phase of the pulse induces a strong current flow to stimulate neurons, whereas the dampened return current is insufficient for neuronal activation. In contrast, biphasic TMS allows energy to oscillate between the capacitor and coil without attenuation, resulting in a polarity switch of the induced current. Both the initial and the reversed current are strong enough to stimulate neurons, but the second is stronger, longer and stimulates different neural elements than the initial one (Groppa et al., 2012). Thus, monophasic TMS presumably stimulates neurons more selectively than biphasic TMS, which induces a more complex pattern of activation (Di Lazzaro et al., 2001).

The intensity of the induced current diminishes proportionally with the square root of the distance from the coil to a depth of up to 1/coil diameter. Thus, cortical regions directly below the coil as well as more distant neurons are affected through transsynaptic interactions (Antal et al., 2022). Consequently, TMS does not exclusively excites one specific but multiple neuronal structures. However, the spatial relationship with the induced electric field, and therefore susceptibility to stimulation, is different for each structure (Siebner et al., 2022).

Depth, precision and focality of the simulation are closely tied to the shape of the inducing coil. Figure-of-eight coils excel in providing focal stimulation, while circular coils are better suited for broader and deeper stimulation (Groppa et al., 2012). Nonetheless, the depth penetration of TMS is limited due to the attenuation of the induced electromagnetic field, and neurons within deep cortical structures (e.g. the thalamus) cannot be excited using TMS. In contrast, TMS is well suited to stimulate cortical areas located close to the induced electromagnetic field at the hemispherical surface, such as the primary motor cortex (M1) (Groppa et al., 2012). When TMS is applied to M1 with intensities sufficient to evoke action potentials, transsynaptic activation spreads along the corticospinal tract, triggering a response in the target muscle (Groppa et al., 2012; Neva et al., 2020). Using surface electromyography

(EMG), the induced muscle response can be recorded, which is called motor evoked potential (MEP). Typically, the TMS-evoked amplitude of the MEP is used to operationalize corticospinal excitability (Klomjai et al., 2015). The MEP also represents a positive control of successful stimulation and allows individual adjustment of the stimulation site and intensity, which is an advantage compared to stimulation of other brain areas (Cooke & Bliss, 2006). The mechanisms of TMS of the M1 and the typical MEP waveform are displayed in *Figure 3*.

Figure 3. *Mechanisms of TMS of the M1 and MEP characteristics.*



Note. Tangential application of the TMS coil over M1 induces a magnetic field that passes through the skull and dura and causes an electric field within the cortex in the opposite direction. This activates cortical interneurons, which synapse on pyramidal neurons, in turn synapsing on spinal motor neurons. A peripheral nerve transmits the signal to the target muscle in the contralateral side. Muscle activation in the target muscle can be recorded via EMG. The amplified EMG signal is displayed on a computer screen for quantification of MEP amplitude and CML. Directly following MEP induction, voluntary EMG activity is suppressed, which is referred to as 'cortical silent period' (CSP). Adapted from Neva et al. (2020), Klomjai et al. (2015), Groppa et al. (2012), and Numssen et al. (2021).

While TMS is typically used to assess corticospinal excitability (Groppa et al., 2012), rTMS can be used to modulate it (Neva et al., 2020), emulating LTP and LTD-like mechanisms through repetitive single-pulse application on the same brain region (Antal et al., 2022). In M1, experience-dependent neuroplasticity is typically assessed by measuring the change in the peak-to-peak MEP amplitude after applying a plasticity-inducing protocol (Jannati et al., 2023).

Various rTMS protocols can lead to persistent changes in cortical excitability, depending on factors such as intensity, frequency, and number of stimuli applied, with frequency playing a crucial role. High-frequency rTMS protocols (≥5 Hz) have been shown to produce LTP-like plasticity, whereas low-frequency rTMS protocols (<5 Hz) induce plasticity similar to LTD (Ziemann et al., 2008). In line with the greater selectivity of monophasic over biphasic TMS (Di Lazzaro et al., 2001), research indicates that the modulation of cortical excitability is stronger using monophasic compared to biphasic rTMS (Arai et al., 2007; Nakamura et al., 2016; Sommer et al., 2002; Taylor & Loo, 2007). Biphasic pulses may activate a wide range of interneurons, potentially resulting in a balancing of inhibitory and facilitatory effects among them. Monophasic pulses on the other hand may allow for a more effective summation of synaptic efficacy (Nakamura et al., 2016). Consistent with the BCM, both suppression of LTP and enhancement of LTD after priming with high-frequency stimulation, suggestive of metaplasticity, have been observed using different rTMS protocols (Hamada et al., 2008; Iyer et al., 2003; Ragert et al., 2009).

In the following, one of the newest rTMS protocols will be described in more detail.

1.5.2.1. Quadripulse stimulation (QPS)

In QPS, four monophasic TMS pulses of the same intensity are applied as one burst from a single coil. One of these bursts is given every 5 sec for 30 min, resulting in a total of 360 bursts and 1,440 pulses (Hamada et al., 2007). High-frequency stimulation induces LTP-like effects, whereas low-frequency stimulation induces LTD-like effects, with preferred inter-stimulus intervals of 5ms (QPS-5) and 50ms, respectively (Matsumoto & Ugawa, 2020). Stimulation intensity is set at 90% of the active motor threshold, i.e., the minimum stimulation intensity required to induce a motor response in a contracted muscle (Hamada et al., 2007).

As described above, monophasic TMS is more powerful than biphasic TMS, as it activates more homogenous groups of neurons (Di Lazzaro et al., 2001) and its activated pathway of intervention matches the pathway of measurement (Huang et al., 2017). In line with this, monophasic QPS induces longer after-effects compared to biphasic QPS (Nakamura et al., 2016) and other rTMS protocols (Hamada et al., 2007). The lower intra- and inter-individual variability of its effects may represent an advantage of QPS over other rTMS protocols (Nakamura et al., 2016; Simeoni et al., 2016; Tiksnadi et al., 2020). However, the responder

rate was only slightly higher compared to other protocols in one of these studies (Simeoni et al., 2016), calling for further investigation of the magnitude and consistency of this effect.

Importantly, QPS selectively modulates excitatory circuits (Hamada et al., 2008), which presumably play an important role in the pathophysiology of MS due to their neurotoxic effect (Kuzmina et al., 2020). In contrast, other excitatory TMS protocols also modulate inhibitory networks (Groiss et al., 2012; Hamada et al., 2008).

1.6. Synaptic plasticity in patients with MS

As outlined in the previous sections, neuroplasticity may be an important factor influencing MS disease progression, potentially explaining the 'clinico-radiological paradox' and differences between disease types. Synaptic plasticity is one of the earliest events in a cascade of functional and structural reorganization processes and is non-invasively assessable using rTMS. RTMS-induced plasticity may, therefore, be a promising biomarker of disease progression in patients with MS.

Despite promising results concerning the prognostic value of LTP-like plasticity in predicting dementia (Di Lorenzo et al., 2020), it has not yet been investigated in the context of MS disease progression. Only one study comparing LTP-like plasticity in patients with RRMS and PPMS indicated that lower levels of LTP-like plasticity may be associated with disease progression (Mori et al., 2013). Furthermore, few studies have compared the level of LTP- or LTD-like plasticity between patients with MS and HCs prior to this thesis. The results are summarized in *Table 1*.

Table 1. *Summary of rTMS studies comparing synaptic plasticity between patients with MS and HCs.*

Disease type	LTP-like		LTD-like	
	Altered	Normal	Altered	Normal
RRMS (stable)	Conte et al. (2016),	Zeller et al. (2010), Mori et al. (2013)	Mori et al. (2013)[a]	Zeller et al. (2012)
RRMS (relapsing)			Wirsching et al. (2018)	
RRMS (mixed)			Mori, Nisticò, et al. (2014)	
PPMS	Mori et al. (2013)		Mori et al. (2013)	

Note. [a] Interpretation of altered plasticity is open to debate. The protocol has been described to induce either LTP or LTD-like effects in HCs. In this study, it induced LTP-like effects in RRMS patients but LTD-like effects in HCs.

In addition to the studies summarized in Table 1, a study exploring effects of treatments or cerebrospinal fluid markers on the degree of synaptic plasticity in patients with MS indicated

that the degree of plasticity is a relevant factor in MS (Mori et al., 2012). However, based on this study, potential differences between HCs and patients with MS, variations among patients with different disease types, and its prognostic significance remain unknown.

In the context of MS relapses, sustained LTP-like plasticity has been linked to clinical recovery. Patients with higher LTP-like plasticity during relapse showed better clinical recovery three months later compared to patients with lower plasticity levels (Mori, Kusayanagi, et al., 2014). Additionally, patients with gadolinium-enhancing lesions in brain imaging, suggestive of active inflammation, presented lower levels of LTP-like plasticity than patients without gadolinium-enhancing lesions (Mori et al., 2012). Conversely, no association between synaptic plasticity during relapse and clinical recovery was found when investigating LTD-like plasticity (Wirsching et al., 2018). Using this inhibitory protocol, LTP-like effects were observed during relapse but not during recovery three months later. This reversal in the induced plasticity direction may signify compensatory metaplastic effects during relapse (Wirsching et al., 2018). However, this remains speculative, as the LTD-inducing protocol did not induce any LTD-like effects in HCs and highly variable effects of this protocol have been described previously (López-Alonso et al., 2014; Strube et al., 2015).

Prior to this thesis, two studies suggested an association between cognitive impairment and lower levels of LTP-like plasticity (Mori et al., 2012; Mori et al., 2011). However, sample size (N=21) was rather low in one of these studies (Mori et al., 2011). In the other study, both LTP-like plasticity and cognitive performance improved after pharmacological treatment, but their association was not directly investigated (Mori et al., 2012).

Collectively, these studies propose that rTMS-induced plasticity holds promise as a tool to elucidate the ‘clinico-radiological paradox’ and may serve as a biomarker of disease progression. However, they are constrained by the high variability and substantial non-responder rates associated with the implemented rTMS protocols (Guerra et al., 2017), which may also account for the conflicting results among studies.

1.7. Aim of this thesis

As outlined in the preceding chapter, research on rTMS-induced plasticity in patients with MS is scarce and revealed inconclusive results. Therefore, the aim of this thesis was to evaluate synaptic plasticity using QPS, potentially inducing plasticity more effectively with reduced variability compared to previous protocols (Nakamura et al., 2016; Simeoni et al., 2016; Tiksnadi et al., 2020). Due to its selective modulation of the excitatory glutamatergic network and given the presumed significance of this network in MS pathophysiology (Hamada et al., 2008; Kuzmina et al., 2020), QPS may be better suited to measure synaptic plasticity in MS than other rTMS protocols.

Prior research predominantly focused on individuals with RRMS, with minimal investigation into patients with PMS, apart from a single study which examined only 12 patients with PPMS (Mori et al., 2013). Although some studies evaluated the prognostic value of rTMS during acute relapse for clinical recovery (Mori et al., 2012; Mori, Kusayanagi, et al., 2014; Wirsching et al., 2018), the prognostic value of synaptic plasticity regarding disease progression has not yet been investigated longitudinally.

In the four studies composing this thesis, neuropsychological and electrophysiological experiments were conducted to explore the link between QPS-induced plasticity and motor and cognitive functions, both cross-sectionally and longitudinally. The primary aim was to investigate QPS-induced plasticity as a potential biomarker for predicting disease progression. The following chapters outline the objectives, hypotheses, and key findings of each study.

2. Studies included in this thesis

This thesis is based on the following studies:

Study 1: Balloff, C.,[1] Penner, I.-K.,[1] 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.,[2] & Albrecht, P.[2] (2022). The degree of cortical plasticity correlates with cognitive performance in patients with Multiple Sclerosis. *Brain Stimulation, 15*(2), 403–413. https://doi.org/10.1016/j.brs.2022.02.007

Study 2: Balloff, C., Novello, S., Stucke, A.-S., Janssen, L.K., Heinen, E., Hartmann, C.J., Meuth, S.G., Schnitzler, A., Penner, I.-K.[2], Albrecht, P.[2], & Groiss, S.J.[2] (2023). Long-term potentiation-like plasticity is retained during relapse in patients with Multiple Sclerosis. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology, 155*, 76–85. https://doi.org/10.1016/j.clinph.2023.07.013

Study 3: Balloff, C.,[1] Albrecht, P.,[1] Stucke, A.-S., Scala, L., Novello, S., Hartmann, C. J., Meuth, S. G., Schnitzler, A., Penner, I.-K.,[2] & Groiss, S.J.[2] (2023). The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis. *Frontiers in Neurology, 14,* Article 1266225. https://doi.org/10.3389/fneur.2023.1266225

Study 4: Balloff, C., Janssen, L.K., S., Hartmann, C. J., Meuth, S.G., Schnitzler, A., Penner, I.-K.,[2] & Albrecht, P.[2] (2024). *Predictive value of synaptic plasticity for functional decline in patients with multiple sclerosis* [Manuscript submitted for publication].

[1] Shared first authorship.
[2] Shared last authorship.

The experimental design, methods, as well as the results of each study are detailed in the attached articles (appendix B). Consequently, the following sections will offer only concise summaries. All studies focused on synaptic plasticity measured at M1, which was operationalized as MEP amplitude change at the relaxed first dorsal interosseous muscle of the right hand following QPS-5 using a figure-of-eight coil.

2.1. Study 1 – Synaptic plasticity in RRMS and its association with cognitive performance

The objectives of the first study were 1) to explore the correlations between QPS-induced plasticity and performance in two frequently affected cognitive domains, namely IPS and visuospatial short-term learning and memory (Chiaravalloti et al., 2013; Deluca et al., 2004; Chiaravalloti & DeLuca, 2008) in patients with RRMS and HCs, and 2) to compare the degree of plasticity between both groups. The hypotheses were: a) positive correlations between the degree of QPS-induced plasticity and cognitive performance, and b) reduced plasticity in RRMS patients compared to HCs.

In line with hypothesis a), better performances on the Symbol Digit Modalities Test (SDMT; Smith (1982)), measuring IPS, and the Brief Visuospatial Memory Test-Revised (BVMT-R; Benedict (1997)) measuring visuospatial short-term learning and memory, were associated with higher levels of plasticity in 63 patients with RRMS. Both associations retained significance after controlling for potential confounding factors, such as the MEP latency, presumably representing, at least partially, the integrity of the corticospinal tract. Further, plasticity was significantly reduced in patients with cognitive impairment compared to those with preserved cognitive function, and the extent of plasticity served as a distinguishing factor between these two patient groups. Exploratory analysis revealed negative correlations of QPS-induced plasticity with MEP latency, age, and the Expanded Disability Status Scale (EDSS; Kurtzke (1983)).

The overall RRMS patient cohort did not exhibit diminished plasticity when compared to HCs (n=55), refuting hypothesis b). No association between any functional outcome and the degree of QPS-induced plasticity was observed in HCs (Balloff et al., 2022).

2.2. Study 2 – Synaptic plasticity during acute relapses

The objectives of the second study were 1) to examine QPS-induced plasticity in patients with MS during acute relapses compared to stable MS patients and HCs, and 2) to assess its functional significance. For the first objective, no specific hypothesis was posited regarding the direction of a potential group difference due to ambiguous previous research findings. For the second objective, it was anticipated that individuals with complete recovery would exhibit higher levels of QPS-induced plasticity compared to those with partial recovery,

and conversely, individuals with no recovery would present lower levels of plasticity compared to those with partial recovery.

Synaptic plasticity was induced by QPS-5 in all groups, and its degree did not differ significantly between acute relapsing MS patients, stable MS patients, and HCs (n=18 per group). Most patients showed at least partial symptom recovery three months after relapse, while only three patients experienced complete recovery and another three patients showed no recovery at all. Therefore, the functional relevance of baseline synaptic plasticity for recovery (objective 2) could not be analyzed. Exploratory analysis revealed that relapsing patients with motor disability exhibited significantly higher plasticity than those without motor disability (Balloff, Novello, et al., 2023).

2.3. Study 3 – Synaptic plasticity in PMS and its association with motor performance

The objectives of the third study were 1) to compare QPS-induced plasticity levels among different MS subtypes and HCs, and 2) to explore the association between plasticity levels and motor and cognitive functions. Considering results from study 1, the hypotheses were: a) reduced plasticity in patients with PMS but not RRMS compared to HCs, and b) positive correlations between the degree of QPS-induced plasticity and cognitive and motor performance in patients with MS, irrespective of the disease type.

Contradicting hypothesis a), no significant differences were found in QPS-induced cortical plasticity between 34 patients with PMS, 30 matched HCs, and 30 matched patients with RRMS. Regarding hypothesis b), no correlations with functional outcomes were revealed for patients with PMS. Exploratory analyses revealed that correlations between induced plasticity and both motor and cognitive functions were observed only in patients with intact corticospinal tract integrity and that QPS-induced plasticity was significantly reduced in patients with damaged corticospinal tract integrity compared to those with intact tracts (Balloff, Albrecht, et al., 2023).

2.4. Study 4 – Prognostic value of synaptic plasticity for disease progression

The fourth study was conducted to elucidate the functional relevance of synaptic plasticity over time. Synaptic plasticity was assessed in a cohort of 80 patients with MS (56 RRMS, 24 PMS), and 69 matched HCs. Annual clinical follow-ups spanning up to five years, with a median follow-up period of two years, were conducted.

The hypotheses were as follows: a) patients with lower baseline plasticity levels experience greater disease progression, defined as functional decline in motor and/or cognitive function and/or progression in EDSS, compared to those with higher baseline plasticity levels. Initially, the second objective of the study was to analyze QPS-induced plasticity changes over time with the hypothesis that b) QPS-induced plasticity diminishes

more rapidly in patients with MS than HCs. However, analyses of hypothesis b) were precluded due to cancellation of annual QPS-5 assessments because of technical defects and the coronavirus disease 2019 (COVID-19) pandemic.

In line with hypothesis a), patients experiencing functional decline in manual dexterity and/or visuospatial learning and memory presented with significantly lower levels of baseline plasticity compared to those without clinically relevant decline in these functions. This association became apparent only when employing linear-mixed-effects models (LMEM) for manual dexterity. For visuospatial learning and memory, receiver-operating characteristic (ROC) analysis underscored the predictive utility of baseline plasticity to differentiate between patients with and without clinically relevant decline at latest follow-up. Cox proportional-hazards models, wherein patients were stratified into low and high baseline plasticity groups through a median split, as well as logistic regression analysis, failed to reveal significant differences between these groups. No significant associations between baseline plasticity and decline in IPS, EDSS, or lower extremity function were found, contradicting hypothesis a).

On average, the patient cohort exhibited no clinically relevant change in any functional outcome over time. Moreover, a comparable number of patients experienced both clinically significant improvement and decline over the observed period, and performance in both the patient group and HCs varied significantly across time points (Balloff et al., 2024).

3. Discussion

The aim of this thesis was to investigate synaptic plasticity using a newer protocol of rTMS-induced plasticity, namely QPS, with presumably lower inter- and intra-individual variability than previously used protocols (Nakamura et al., 2016; Simeoni et al., 2016; Tiksnadi et al., 2020). In the following, the main findings of the four studies included in this thesis will be summarized and put in perspective with the literature. Emphasis will be placed on scrutinizing strengths and limitations with particular attention directed towards the variability of rTMS effects, plasticity mechanisms beyond LTP-like phenomena at M1, additional factors impacting disease progression beyond neuroplasticity, unforeseen circumstances encountered throughout the study, and recommendations for future research endeavors.

3.1. Summary and interpretation of the results

The first study demonstrated that higher levels of QPS-induced plasticity are associated with better cognitive function in patients with RRMS. This aligns with previous research reporting differences in LTP induced by theta burst stimulation (TBS) between cognitively preserved and cognitively impaired MS patients, alongside concurrent improvement in intermittent TBS and cognitive performance (Mori et al., 2012; Mori et al., 2011).

Additionally, no differences were observed in the global level of QPS-induced plasticity compared to HCs (Balloff et al., 2022). This finding is consistent with prior studies indicating preserved TMS-induced plasticity using other rTMS protocols (Mori et al., 2013; Zeller et al., 2010), but contradicts another study reporting reduced plasticity in patients with MS (Conte et al., 2016). During this thesis, two additional studies utilizing intermittent TBS have emerged, reporting altered LTP-like plasticity in stable RRMS patients (Baione et al., 2020; Stampanoni Bassi et al., 2023).

Studies using different rTMS protocols are hardly comparable. Yet, our finding of reduced LTP-like plasticity in patients with cognitive impairment suggests that reduced plasticity in other studies could be attributed to cognitive impairment within these cohorts. Indeed, the cohort studied by Conte et al. (2016) performed worse on the SDMT than our cohort, which could account for the divergent results. Unfortunately, no cognitive data were presented in the other two studies reporting altered LTP-like plasticity (Baione et al., 2020; Stampanoni Bassi et al., 2023). Therefore, it remains speculative whether cognitive impairment might explain the different results regarding LTP-like plasticity in stable RRMS.

Although the first study presented initial evidence that QPS-induced plasticity may be of functional relevance, the results were limited to RRMS patients in the remitting phase of the disease and cognitive decline, which is only one of several possible symptoms associated with MS. However, exploratory analysis revealed a negative correlation of QPS-induced plasticity with the EDSS, suggesting that high levels of QPS-induced plasticity are not only associated with better cognitive performance but also lower levels of disability. This indicates that QPS-induced plasticity may be relevant for the clinical status beyond cognition (Balloff et al., 2022).

The second study was conducted to examine QPS-induced plasticity in MS patients during acute relapses compared to stable MS patients and HCs. Additionally, it sought to evaluate its functional relevance for clinical recovery. It was revealed that QPS-induced synaptic plasticity persists during acute MS relapses, and subgroup analyses suggested that stabilizing metaplastic mechanisms may be crucial in preventing motor disability. However, the sample size was rather small requiring further verification of these findings in larger studies. Furthermore, one of the research questions, namely the functional relevance of plasticity for clinical recovery, could not be addressed due to insufficient numbers of patients experiencing null or complete recovery three months after relapse (Balloff, Novello, et al., 2023).

Given that only 33% of relapsing patients presented cognitive impairment in the second study, the first study's observation of preserved plasticity in stable RRMS patients without cognitive impairment may be extended to relapsing RRMS patients without cognitive impairment. However, it has been discussed that increased levels of pro-inflammatory mediators may influence synaptic plasticity (Stampanoni Bassi et al., 2022), potentially promoting hyperexcitability during relapses (Mandolesi et al., 2012; Rossi et al., 2011). Indeed,

hyperexcitability during relapse may have occurred in study 2, given that most relapsing patients had undergone glucocorticoid treatment prior or during study participation and glucocorticoids have been described to suppress LTP (Brandner et al., 2022; Dinse et al., 2017; Park et al., 2015).

A potentially significant role of the concept of metaplasticity during MS relapses is supported by previous research. Unfortunately, detailed information on the functional system scores of relapsing patients presenting with reversed effects of an LTD protocol in a previous study were not disclosed (Wirsching et al., 2018). However, considering the reported EDSS median of 2.0 and predominant relapse symptoms (only n=1 with motor symptoms), this cohort appears comparable to the subgroup of patients without motor disability in study 2. In summary, both studies suggest that stabilizing metaplastic mechanisms may be crucial to prevent clinical (motor) disability during relapse, with synaptic plasticity itself potentially playing a secondary role. However, in another study using a paired associative simulation protocol with an interstimulus interval of 25ms (PAS 25), rTMS-induced plasticity was revealed as an independent predictor of recovery three months after relapse (Mori, Kusayanagi, et al., 2014). Therefore, more research is needed to understand the relevance of synaptic plasticity and metaplasticity for clinical recovery after MS relapses.

The third study was conducted to compare QPS-induced plasticity levels among different MS subtypes and HCs, and to explore the association between plasticity levels and motor and cognitive functions. The results suggest that MEP latency, representing the integrity of the corticospinal tract (Neva et al., 2016), should be considered when examining cortical plasticity in patients with MS, while the MS disease course may be secondary. Although study 1 had revealed a negative association of QPS-induced plasticity with MEP latency, the association between QPS-induced plasticity and cognitive performance remained significant after controlling for MEP latency. Therefore, based on the first study, the importance of the corticospinal tract integrity revealed in study 3, was not expected (Balloff et al., 2022).

The fact that patients of all disease types presented with preserved plasticity compared to HCs contrasts with the assumption that the progressive phase of the disease is marked by an insufficient compensatory reserve to counteract the adverse effects of inflammation and neurodegeneration (Antel et al., 2012). It also seems to contradict the results of a previous study reporting lower levels of LTP-like plasticity in patients with PPMS compared to RRMS (Mori et al., 2013). However, in this study, patients with PPMS exhibited significantly higher MEP latency, indicating involvement of the corticospinal tract in this subgroup. Given the significance of the corticospinal tract suggested in study 3, it is plausible that low levels of LTP-like plasticity in patients with PPMS in the previous study might have been driven by corticospinal tract involvement rather than the specific type of MS. Additionally, intermittent and continuous TBS have been described to yield more variable effects in comparison to QPS

(Guerra et al., 2017; Nakamura et al., 2016; Tiksnadi et al., 2020) and the previous study was conducted on only n=12 patients with PPMS. Considering both the limited sample size and high variability of the implemented protocol, the significant main effect may not represent a systematic difference in the degree of plasticity between disease types but rather an unsystematic effect of measurement variability.

Another study investigating LTP-like plasticity in patients with PMS using a PAS 25 protocol has been published during this thesis, reporting reduced LTP-like plasticity compared to HCs (Stampanoni Bassi et al., 2023). The sample size (n=18) was smaller than in our study (n=34) and motor impairment appeared to be more pronounced, as indicated by longer average completion times for the nine-hole peg test and timed 25-foot walk test compared to our cohort (30 sec and 20 sec vs. 25 sec and 6 sec). This may explain the divergent result compared to study 3.

In summary, the first three studies indicate that QPS-induced plasticity is preserved in patients with MS, regardless of the disease type. Furthermore, it seems to be of functional relevance in patients with intact corticospinal tract. However, these studies were conducted cross-sectionally, precluding any inference of a causal relationship between QPS-induced plasticity and functional parameters. To longitudinally assess the functional relevance of QPS-induced plasticity, the fourth study was conducted.

The degree of baseline plasticity was associated with clinically relevant decline in manual dexterity as well as visuospatial learning and memory over a median follow-up time of two years only when analyzed using LMEM. ROC-analysis also indicated a predictive value of baseline plasticity for detecting subsequent functional decline in visuospatial learning and memory, but not for decline in any other outcome (Balloff et al., 2024). Using LMEM, the baseline level of synaptic plasticity was compared between patients with and without clinically relevant decline, accounting for individual variations in the increase of MEP amplitude following QPS via a random slope. None of the other statistical approaches considered inter-individual variations, which may explain the differing results. Cox proportional-hazards models did not only assess whether but also when an event, i.e. clinically relevant functional decline, occurred. However, due to the relatively small number and short period of follow-ups in this study, the variability in the timing of events was constrained. Furthermore, the limited number of patients experiencing clinically relevant functional decline may have introduced statistical artifacts.

Notably, the patient cohort was, on average, clinically stable throughout the follow-up period. Although some patients experienced clinically relevant functional decline, a comparable number of patients significantly improved. This finding aligns with previous studies showing that both cognitive and physical disability progression occur slowly and vary greatly among individuals. In a study with a five-year follow-up period, cognitive decline was observed in 28%, with more PMS compared to RRMS patients affected (Eijlers et al., 2018). Even in a

study with a follow-up period of 10 years, neither relevant cognitive nor physical disability changes were observed on the group level, with only 24% of the patients experiencing cognitive decline (Pinter et al., 2021). However, depending on the study design, drop-out rate, and baseline characteristics, higher (49%-62%) as well as lower rates (10%) of cognitive decline have been described for the same follow-up period as well (Carotenuto, Costabile, et al., 2022; Damasceno et al., 2020; Katsari et al., 2020).

Reserve mechanisms may only be activated specifically when pathology demands compensatory responses (Sumowski & Leavitt, 2013). This is supported by study 1 and study 3, which both revealed no correlation between QPS-induced plasticity and any functional parameter in HCs. Consequently, the clinical relevance of neuroplasticity in study 4 might have been limited by minimal disease-related activity requiring compensation. However, this interpretation remains speculative given the lack of indicators of disease activity beyond clinical outcomes in study 4.

Interestingly, another study published during this thesis supports the relevance of LTP-like plasticity for manual dexterity. Stampanoni Bassi et al. (2023) reported significant positive correlations between baseline LTP-like plasticity and improvement in the nine-hole peg test after eight weeks of physical therapy in patients with PMS. Although the design of this study is hardly comparable to study 4, both studies indicate that reduced levels of LTP-like plasticity at M1 may correlate with poorer manual dexterity outcomes.

In the following sections, strengths and limitations of the studies composing this thesis will be discussed and recommendations for future research will be provided.

3.2. Strengths and limitations of the studies

The primary strength of these studies resides in their methodological approach. Prior to this thesis, the QPS protocol had never been applied to patients with MS, yielding for an assessment of its usefulness in this group of patients. Utilizing this protocol to evaluate synaptic plasticity in MS patients may offer increased reliability compared to previously employed rTMS paradigms (Nakamura et al., 2016; Simeoni et al., 2016; Tiksnadi et al., 2020). This enhanced reliability may be attributed to the QPS protocol's specificity in selectively influencing excitatory networks (Hamada et al., 2008), which have been identified as pivotal in MS pathology (Groom et al., 2003; Kuzmina et al., 2020; Schirmer et al., 2019). Additionally, prior to this thesis, rTMS experiments conducted in patients with MS had not been analyzed using LMEM, further underscoring the novelty and rigor of this thesis.

Furthermore, except for study 2, the sample sizes of the studies greatly exceeded those of previous investigations. This increase in sample size substantially enhances the robustness and reliability of the findings. Clinical characteristics and their effects on the measure of plasticity were analyzed in detail, potentially limiting confounding effects and improving

comparability with other studies. Lastly, patients with additional neurological or psychiatric diseases were excluded from the studies to further limit the influence of confounding factors.

Despite these strengths, there are some important limitations which need to be considered. Due to the similar clinical and pathophysiological presentation (Lassmann, 2018), patients with PPMS and SPMS were summarized to one group of PMS to increase statistical power. However, there are also substantial differences between both disease types (Antel et al., 2012; Lassmann, 2018). Exploratory analysis did not reveal any differences across groups in study 3 but may have been underpowered due to the small sample size per subgroup (n=14 PPMS patients, n=20 SPMS patients).

Due to the time-consuming method of TMS-stimulation, the Brief International Cognitive Assessment for MS was limited to the SDMT and BVMT-R, excluding verbal learning and memory assessment. This two-test combination is recommended in time-restricted settings due to its high sensitivity to detect cognitive impairment (Baetge et al., 2020) and the high relevance of these cognitive functions for daily living (Campbell et al., 2017). However, assessment of verbal learning and memory could have added important information, as recent evidence suggests that cognitive disability progression primarily occurs in this domain (Katsari et al., 2020). Furthermore, other functional outcomes, e.g. working ability, and executive functioning may be important to explore in future studies as well.

Practice effects, characterized by increases in test scores due to prior exposure to the same or similar neuropsychological measure (Heilbronner et al., 2010), are frequently observed in neuropsychological testing. To mitigate this issue, we utilized alternate forms of the BVMT-R in annual follow-ups. However, identical test materials represent only one facet contributing to practice effects. Other factors include familiarity with the testing environment, procedural learning, regression to the mean, and test sophistication (Bartels et al., 2010). Due to the absence of normative data for alternate forms, the same version of the SDMT was used consistently. In general, practice effects diminish with longer re-test intervals and manifest differently in clinical compared to non-clinical populations (Calamia et al., 2012). The effect of the re-test interval has recently been confirmed in patients with MS. Fuchs et al. (2022) reported that repeated administrations of identical SDMT forms were predictive of enhanced performance, particularly when the time intervals between tests was <2 years. It has, therefore, been suggested that maintaining an unchanged SDMT score through the fifth annual assessment using the same form indicates impairment (Fuchs et al., 2022). Given the recency and unknown clinical relevance of this finding, we decided to use established cut-offs of clinically meaningful change. However, for the SDMT, the threshold of ≥8 points was derived from a study employing a longer re-test interval and less frequent neuropsychological testing compared to our study (Weinstock et al., 2021). Conversely, BVMT-R thresholds stemmed from studies involving shorter intervals of two to four weeks (Benedict et al., 2012).

Furthermore, the considerable number of HCs presenting clinically relevant cognitive decline/improvement according to these thresholds suggests that the established thresholds for reliable change on neuropsychological tests may require adjustment. In summary, clinically relevant cognitive decline may not have been accurately detected in study 4.

Although the EDSS represents an internationally accepted instrument, several aspects have been criticized, e.g. its reliability, sensitivity to change, and ordinal scale level (please refer to Meyer-Moock et al. (2014) for a comprehensive literature review on the validity of the EDSS). Consistent with this, the proportion of patients with clinically relevant decline in EDSS scores in study 4 was nearly equivalent to those demonstrating clinically relevant improvement (24% vs. 19%). This aligns with previous data reporting 21% of patients experiencing improvement compared to 25% worsening over a span of five years (Giovannoni et al., 2021).

Given the considerable diversity in stimulation protocols, target muscles, and study populations, varying recommendations exist for the number of averaged trials required to ensure reliable assessments of MEPs (Bashir et al., 2017; Biabani et al., 2018; Cavaleri et al., 2017; Goldsworthy et al., 2016). In all studies incorporated in this thesis, 12 MEPs were averaged to uphold a streamlined protocol, avoid participant fatigue and fluctuations of attention within the experiment. Nonetheless, it is essential to acknowledge that this number of averaged MEPs falls at the lower end of the recommended spectrum and that increasing the number of averaged MEPs might have enhanced the reliability of our findings.

Lastly, the absence of imaging data prevented the analysis of how (sub)cortical lesions might have affected MEP latencies. Possibly, prolonged MEP latencies were not only the result of impaired corticospinal tracts but also of abnormalities in the motor cortex. Furthermore, the level of underlying disease-activity remained unknown.

In the following, the most complex limitations will be discussed in more detail.

3.2.1. Variability of rTMS effects

Inter-individual variability of rTMS protocols is an important limitation of this method. Low expected responder rates, i.e. patients showing the expected increase/decrease of MEP amplitude, have been particularly described for other rTMS protocols than QPS, i.e. intermittent and continuous TBS, and PAS 25 (Hamada et al., 2013; Lahr et al., 2016; López-Alonso et al., 2014). Studies comparing QPS with these protocols are rare but indicate lower inter- and intra-individual variability of QPS (Nakamura et al., 2016; Simeoni et al., 2016). Nonetheless, the effect of QPS has been described to be influenced by voluntary movement of the target muscle following QPS intervention (Kadowaki et al., 2016), as well as caffeine intake (Hanajima et al., 2019). Further factors described to impact the effects of other rTMS protocols, e.g. age, attention, sex, genetics, aerobic exercise, pharmacological influences, and time of day (Ridding & Ziemann, 2010) may influence the effects of QPS as well. Most of these

factors, e.g. age, sex, and pharmacological influences, were controlled for in our studies. In all experiments, attention was aimed to be held at a constant level to reduce variation in the outcome by instructing the participants to count the number of stimuli. However, this task requires only small levels of attention, and it was not tested throughout the experiment. Other researchers incorporated more complex tasks requiring attention (Wirsching et al., 2018), thus potentially ensuring higher levels of attention throughout the experiment.

Fatigue is a common symptom of MS (Oliva Ramirez et al., 2021), which may have further contributed to fluctuating levels of attention (Hanken et al., 2015). In study 1, both cognitively impaired and non-impaired MS patients reported comparable levels of fatigue, yet their plasticity levels differed. This indicates that the relationship between QPS-induced plasticity and cognitive performance remained unaffected by fatigue. Additionally, the overall patient group exhibited similar levels of plasticity compared to HCs, although half of the patients experienced at least moderate fatigue. However, fatigue was operationalized using a fatigue questionnaire measuring trait fatigue instead of the level of fatigue during the experiment. Therefore, patients with low scores on the fatigue scale might have still experienced severe fatigue during the experiment and vice versa.

Patients were excluded from the studies if they were using dextromethorphan or any illegal drugs, given their well-documented impact on synaptic plasticity measures (Ridding & Ziemann, 2010). There may, however, be other substances not controlled for in our experiments, e.g. baclofen (McDonnell et al., 2007) and nicotine (Swayne et al., 2009), influencing QPS-induced plasticity. Further, some factors may interact with each other (Ridding & Ziemann, 2010).

Sleep may have influenced not only LTP, but also the functional outcomes in the studies, i.e. cognitive and motor performance (Al-Sharman et al., 2021; Braley et al., 2016). Since this factor was not controlled in any of the studies incorporated in this thesis, random noise not associated with plasticity may have been induced.

Importantly, studies regarding the inter-individual variability of QPS are based on HCs only. However, factors contributing to the variability of QPS may be particularly important in patients with MS, as neuroinflammation and neurodegeneration may increase inter- as well as intra-individual variability compared to HCs (Huang et al., 2017). For instance, research has indicated that pro-inflammatory mediators can impact synaptic functioning and plasticity, potentially promoting hyperexcitability (Stampanoni Bassi et al., 2022). Furthermore, individuals with MS were on various disease-modifying therapies and symptomatic medications, potentially influencing cortical excitability. Notably, stabilizing effects of disease-modifying therapies on cortical excitability have been reported in patients with PMS (Ayache et al., 2015).

Reports on the variability and reliability of QPS are limited to the same Japanese research group and one laboratory in the UK. Although the efficacy of QPS-5 was still reported to be higher compared to that of other rTMS protocols, the UK laboratory reported lower expected responder rates than the Japanese laboratories (Nakamura et al., 2016; Simeoni et al., 2016; Tiksnadi et al., 2020). Therefore, potentially confounding effects of ethnicity and/or genotype cannot be excluded and confirmation of the efficacy of QPS in other laboratories is needed (Matsumoto & Ugawa, 2020). While this lies beyond the scope of this thesis, the data gathered from HCs throughout the studies may prove valuable for such endeavors in the future.

Lastly, rTMS was applied using a hand-held coil and the motor hot spot was determined manually. Despite the well-trained staff, minor variations in coil position during the experiment and/or suboptimal spatial precision in motor hot spot determination may have occurred. This is critical given that even minor variations in coil placement on the skull can result in changes to the cortical area being stimulated (Richter et al., 2013). However, the gold standard to improve spatial precision and constant coil position, namely neuro navigated TMS, requires individual MRI data (Jannati et al., 2023). Given the extensive study protocol, adding a time- and resource-intensive MRI assessment did not seem feasible.

In addition to these ‘external’ factors influencing the effects of QPS-5, the activity of the stimulated cortex at the time of stimulation can impact the effects of the stimulus (Zrenner & Ziemann, 2023). Brain activity can be detected using electroencephalography (EEG) and is primarily characterized based on rhythm and frequency (Feyissa & Tatum, 2019). An 8-14 Hz alpha rhythm within the somatosensory or motor cortex is often referred to as ‘sensorimotor mu-(alpha) rhythm’ (Thies et al., 2018). It has been shown, that the negative vs. positive EEG-peak of this ‘sensorimotor mu-rhythm’ is associated with high vs. low excitability of corticospinal neurons (Zrenner et al., 2018). In addition to the phase of the EEG oscillation, other characteristics of brain activity, exceeding the scope of this thesis, influence brain excitability (Zrenner & Ziemann, 2023).

In the studies integrated in this thesis, a fixed stimulation pattern and intensity were applied irrespective of the ongoing brain activity at the stimulated site, representing an ‘open-loop brain stimulation’ approach. Consequently, the specific state of network excitability (high or low) at the time of stimulation remains undisclosed for each participant, although potentially influencing the effects of QPS-5. In contrast, ‘closed-loop brain stimulation’ integrates neural activity at the stimulated cortical area through a first-order trigger and a second-order update function. These functions determine whether and with what parameters to stimulate, and evaluate the induced effects, respectively (Zrenner & Ziemann, 2023). Although this may significantly improve the effects of QPS-5 and other rTMS protocols in the future, ‘closed-loop

brain stimulation' has not yet been incorporated into research on plasticity induction due to various technical challenges (Zrenner & Ziemann, 2023).

3.2.2. Plasticity beyond LTP-like plasticity at M1

As outlined in chapter *1.4. Neuroplasticity and cortical plasticity*, neuroplasticity is a multifactorial process with various factors unfolding on different temporal scales. This thesis focused on synaptic plasticity, representing rapid-onset mechanisms of neuroplasticity (Matsumoto & Ugawa, 2020; Zeller & Classen, 2014), as it is most easily studied. It is believed that these rapid-onset mechanisms constitute the initial phases of more slowly unfolding long-term processes of plasticity (Zeller & Classen, 2014). However, assessing only these rapid-onset mechanisms may not be sufficient to capture all aspects of neuroplasticity, e.g. structural changes. Further, only LTP-like plasticity was investigated, since MS has been associated specifically with alterations in the glutamatergic network (Kuzmina et al., 2020). However, LTD-like plasticity may also be relevant, as inhibitory circuits may be involved in MS as well (Nantes et al., 2016). In fact, rather than any of these two types of plasticity alone, their interplay may be critical. Consistent with this, study 2 indicated that stabilizing metaplasticity during relapses may be more important than LTP-like plasticity in preventing motor disability.

Furthermore, it has recently been suggested to not only assess synaptic plasticity at M1, but also at association motor-related areas (Neva et al., 2020). These areas, e.g. premotor cortices, can take over motor functions of M1 (Frost et al., 2003) and can influence and contribute to M1 corticospinal outputs (Neva et al., 2020).

It is important to keep in mind that neuroplasticity is not confined to a single location and can manifest differently across various locations and cortical levels. Therefore, qualitative and quantitative differences in synaptic plasticity may exist between cortical and subcortical levels (Sharma et al., 2013). Consequently, although the studies included in this thesis did not identify significant differences in LTP-like plasticity between patients with MS and HCs at M1, other processes of plasticity and/or plasticity at different levels of the neuroaxis may be altered in these patients.

Synaptic plasticity was operationalized as the change in MEP amplitude in the relaxed first dorsal interosseous muscle of the right hand only, independent of handedness and clinical disability. This decision was made to limit the time of examination to a minimum, but hemispheric differences of QPS-induced plasticity may also occur (Chaves et al., 2021).

Another important aspect to consider is that LTP- and LTD-induction through rTMS in a controlled laboratory setting signifies a 'passive' form of plasticity, requiring no action of the participant, apart from maintaining a state of relaxation. However, in vivo, plasticity is induced solely through activity-dependent mechanisms, i.e. (repeated) action by the individual. Therefore, the degree of plasticity assessed during an rTMS-experiment may be interpreted

as the synaptic plasticity ‘potential’. In vivo, individuals with high experimental synaptic plasticity potential may not effectively utilize it, possibly experiencing faster clinical decline compared to those with lower levels of experimental synaptic plasticity, who fully leverage their potential.

In addition to neuroplasticity, other factors may have influenced disease progression in study 4, which will be discussed in the following.

3.2.3. Other factors influencing disease progression

Different types of neuroplasticity react upon changes in the CNS. However, disease progression in patients with MS may also be prevented by premorbid reserve, allowing pathological CNS changes to occur without the need of compensation. The concept of reserve was first introduced by Katzman et al. (1988) following a postmortem examination comparing the brains of individuals with clinical symptoms of dementia during their lifetime to those without such symptoms. Despite both groups exhibiting high levels of Alzheimer’s Disease pathology, the brains of clinically non-demented individuals revealed greater number of neurons and higher brain weight compared to those with clinical symptoms. Since the discovery of this initially passive concept of reserve, ongoing refinement incorporated active aspects. For instance, according to the concept of cognitive reserve, individuals demonstrating more efficient utilization of brain networks or an enhanced ability to engage alternative brain networks when required possess higher reserve (Stern, 2002).

This is in line with the proposition that clinical MS symptoms evolve as a consequence of a ‘network collapse’ (Schoonheim et al., 2022). A more robust network prior to disease onset potentially delays its collapse. Consequently, connectivity across the entire brain may be at least as important as synaptic plasticity in patients with MS. For example, individuals with higher educational attainment or those involved in intellectually demanding activities likely possess a network of diverse pathways. In the case of damage to regions associated with these activities, the impact may be less severe, as alternative pathways can compensate without necessitating reinforcement of synaptic plasticity. Conversely, individuals with high levels of physical activity may exhibit greater resilience to damage in motor areas.

This aspect remains unexplored in the studies presented in this thesis, primarily due to the absence of a validated German assessment tool. However, (premorbid) cognitive engagement was rather high in the longitudinal cohort, with a median of 15 years of education, potentially contributing to the low prevalence of cognitive decline in this cohort.

Disease-modifying therapies have already been acknowledged as potential confounding factors affecting cortical excitability in chapter *3.2.1. Variability of rTMS effects*. However, the primary objective of these therapies is to decelerate disease progression, thereby potentially influencing the primary outcome of study 4. Given the diverse array of

disease-modifying therapies and the limited sample size in the studies, controlling for the effects of these therapies was not feasible.

Moreover, study 4 did not account for potential rehabilitation interventions or personal cognitive/physical training between follow-up assessments. Recent findings suggest that patients with MS, especially those with RRMS, the predominant subgroup in study 4, may experience positive outcomes from such interventions (Chen et al., 2021). This omission should be considered when interpreting the results.

3.2.4. Unforeseen circumstances throughout the study period

Initially, the annual follow-up plan included repeated assessments of QPS-5. However, due to unforeseen circumstances such as the COVID-19 pandemic and technical defects of the TMS coils and signal amplifier, the application of QPS-5 was halted for 12 months in total. To allocate resources towards recruiting new participants after the repairs and easing of pandemic restrictions, annual QPS-5 follow-ups were discontinued. Therefore, analysis of hypothesis b) was precluded in study 4.

To ensure clinical follow-ups, video-based neuro(psychological) testing was introduced for follow-up assessments. Prior research has demonstrated that remote administration of the SDMT produces results comparable to those of in-person testing, thereby validating its suitability for virtual assessment of IPS (Barcellos et al., 2021; Eilam-Stock et al., 2021; Rogers et al., 2023). Limited research exists on the validity of virtual assessment using the BVMT-R, with one study suggesting higher scores in remote settings (Rogers et al., 2023). Fortunately, remote assessment was included as the latest follow-up in study 4 only for four participants (two RRMS patients and two HCs).

During the mandatory break of the studies, a research project investigating neuro(psycho)logical changes in patients with COVID-19 was initiated (refer to appendix C for list of publications). Findings from this project and other studies indicate that COVID-19 can lead to neurological manifestations (Balloff, Bandlow, et al., 2023; Groiss et al., 2020; Misra et al., 2021), some of which may persist for months after the acute phase of the disease (Costa et al., 2023; Hastie et al., 2023; Legler et al., 2023). Consequently, COVID-19 might have influenced the outcome measures in study 4. However, recent evidence suggests that COVID-19 did not affect disease activity, disability progression, or cognitive function in patients with MS (Montini et al., 2024). This is further supported by meta-analyses, which consistently found no association between COVID-19 and an increase in EDSS scores or higher risks of relapses (Aghajanian et al., 2024; Seyedmirzaei et al., 2024).

3.3. Implications and recommendations for future research

Despite the limitations addressed in the previous chapters, this thesis provides important insights into QPS-induced plasticity in patients with MS, which had not been investigated beforehand.

The associations of QPS-induced plasticity with functional outcomes imply that promotion of synaptic plasticity may be a promising tool to prevent clinical deterioration and/or to use as a rehabilitation effort. This idea has already been proposed by Stampanoni Bassi et al. (2022). However, the fact that corticospinal tract integrity was revealed as a prerequisite for these associations highlights the need to report detailed clinical characteristics in research on rTMS-induced plasticity in patients with MS. Although results of studies using different rTMS protocols are hardly comparable, consideration of the clinical characteristics may help to integrate the conflicting results of previous studies (see chapter *1.6. Synaptic plasticity in patients with MS*). Further, they warrant attention in the conceptualization and interpretation of future studies.

It is crucial to acknowledge that numerous factors may impact the reliability of QPS-induced plasticity in patients with MS. The decision on which factors to control for is important and should consider the strains imposed on the patients. Although incorporation of 'closed-loop brain stimulation' into research on plasticity induction has not yet been realized (Zrenner & Ziemann, 2023), this is an important aspect to consider for future studies on QPS-induced plasticity in patients with MS. It may offer insights into interindividual differences in response to QPS and rTMS in general and may, thus, increase the clinical utility of QPS-induced plasticity on the individual level.

Furthermore, LMEM appears to be the best statistical method to capture relevant associations between QPS-induced plasticity and functional outcomes and should be incorporated more frequently in rTMS research.

Applying a complex and time-consuming technique such as QPS to a cohort with limited physical and/or mental resilience is challenging and should not be underestimated. Recruitment of patients for such studies is difficult, potentially resulting in a recruitment bias to highly motivated patients with presumably higher levels of reserve. This specifically applies to patients with PMS, who are typically more severely impaired than patients with RRMS (Engelhard et al., 2022; McGinley et al., 2021), impeding participation in studies with extensive protocols due to exhaustion or mobility issues.

Longitudinal performance of both patients with MS and HCs varied considerably on functional outcomes in study 4. Even when classifying into clinically meaningful decline/improvement based on established cut-offs in MS research considerable variation occurred. This highlights the need to consider that both cognitive and motor performance are volatile and influenced by several (un)controllable factors. Both clinicians and researchers

should keep in mind that performance on any of these tests only represents the performance on a specific day at a specific time. Therefore, further research is needed on reliable change in both HCs and patients with MS with annual neuro(psycho)logical assessments.

To reflect the complexity of synaptic plasticity and to shed light on metaplastic effects, future research should investigate both LTP- and LTD-like plasticity in the same cohort. Additionally, the minimum number of required MEPs per trial to receive a reliable estimate of MEP amplitude requires further research not only in HCs but also in clinical cohorts. Currently, different recommendations exist (Chang et al., 2016; Goldsworthy et al., 2016) and, in general, standard errors reduce with increasing sample size. However, in clinical cohorts it is critical to limit the protocol to a minimum without impeding measurement reliability.

4. Conclusion

This thesis conducted a comprehensive examination of the functional relevance of QPS-induced LTP-like plasticity in patients with MS. The results indicate preserved LTP-like plasticity across all MS subtypes and disease activity states. It was revealed that the consideration of clinical characteristics within patient cohorts is pivotal in synaptic plasticity research in MS, given that associations between LTP-like plasticity and cognitive and motor performance may predominantly manifest in individuals with intact pyramidal tract integrity. Furthermore, the results of this thesis suggest that the degree of LTP-like plasticity may be associated with functional decline. However, the significance of these findings warrants replication in other cohorts and further exploration over longer follow-up periods to ascertain their robustness and generalizability.

Overall, this thesis enhances our understanding of synaptic plasticity in patients with MS while also addressing the methodological challenges associated with its assessment. While QPS-induced plasticity may emerge as an additional facet in unraveling the ‘clinico-radiological paradox’ in patients with MS, it cannot yet be established as an independent biomarker for predicting disease progression at the individual level due to various methodological challenges.

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Appendix A: 2017 McDonald criteria for diagnosis of multiple sclerosis

Table A1. *2017 McDonald Criteria for diagnosis of multiple sclerosis in patients with an attack at onset*

Clinical presentation	Additional data needed for MS diagnosis
≥ 2 clinical attacks and objective clinical evidence of ≥ 2 lesions; or ≥2 clinical attacks and objective clinical evidence of 1 lesion and clearcut historical evidence of a prior attack involving a lesion in a distinct anatomic location	None
≥ 2 clinical attacks and objective clinical evidence of 1 lesion	Dissemination in space, demonstrated by an additional clinical attack implicating a different CNS site *OR* Demonstration of dissemination in space by MRI
1 clinical attack and objective clinical evidence of ≥ 2 lesions	Dissemination in time, demonstrated by a second clinical attack *OR* Demonstration of dissemination in time by MRI *OR* Demonstration of cerebrospinal-fluid-specific oligoclonal bands
1 clinical attack and objective clinical evidence of 1 lesion	Dissemination in space and time, demonstrated by: *For dissemination in space:* A second clinical attack implicating a different CNS site *OR* Demonstration of dissemination in space by MRI *For dissemination in time:* A second clinical attack *OR* Demonstration of dissemination in time by MRI *OR* Demonstration of cerebrospinal-fluid-specific oligoclonal bands

Note. MS= multiple sclerosis; CNS= central nervous system; MRI= magnetic resonance imaging. Adapted from “Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria” by Thompson et al. (2018).

Table A2. *2017 McDonald Criteria for primary progressive multiple sclerosis (PPMS)*

Clinical presentation	Additional criteria
One year of disability progression (retrospective or prospectively determined) independent of clinical relapse	2 out of the following: 1) ≥ 1 T2-hyperintense lesions in ≥ 1 areas in the brain characteristic of MS (periventricular, cortical/juxtacortical or infratentorial) 2) ≥ 2 T2-hyperintense lesions in the spinal cord 3) Presence of cerebrospinal-fluid-specific oligoclonal bands

Note. MS= multiple sclerosis. Adapted from “Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria” by Thompson et al. (2018).

Appendix B: Original research articles

This thesis is based on the following original research articles:

Study 1 – Synaptic plasticity in RRMS and its association with cognitive performance

Balloff, C.,[3] Penner, I.-K.,[3] 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.,[4] & Albrecht, P.[4] (2022). The degree of cortical plasticity correlates with cognitive performance in patients with Multiple Sclerosis. *Brain Stimulation, 15*(2), 403–413. https://doi.org/10.1016/j.brs.2022.02.007

Conceptualization & methodology: I defined the research question and analysis plan in consultation with S.J. Groiss, P. Albrecht, and I.-K. Penner based on the experimental design, which was created by S.J. Groiss, P. Albrecht, and I.-K. Penner.

Investigation & project administration: I supervised recruitment of participants and data collection. I recruited participants and independently performed neuropsychological tests and rTMS. I was also responsible for data curation. Project administration was performed by me, S.J. Groiss, P. Albrecht, and I.-K. Penner. S.J. Groiss and P. Albrecht conducted neurological assessments. M. Ma, I. Georgiades and L. Scala recruited participants and conducted neuropsychological tests and rTMS under supervision. N. Troullinakis, J. Graf, D. Kremer, O. Aktas, S.J. Groiss and P. Albrecht contributed to the recruitment of participants.

Formal analysis: I conducted the statistical analyses independently and reviewed them for correctness.

Resources: S.J. Groiss, P. Albrecht, and I.-K. Penner provided funding for this study. Further resources were provided by H.-P. Hartung, S.G. Meuth and A. Schnitzler.

Manuscript: I wrote the initial manuscript, which included all steps from comprehensive literature research to final formulation. I created the figures and tables independently. I coordinated the scientific review process at the journal. During this process, with the assistance of I.-K. Penner, S.J. Groiss, and P. Albrecht, I made revisions. I prepared the final version of the manuscript. All authors critically reviewed the manuscript. I.-K. Penner, S.J. Groiss, and P. Albrecht additionally contributed to the original draft.

[3] Shared first authorship.
[4] Shared last authorship.

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The degree of cortical plasticity correlates with cognitive performance in patients with Multiple Sclerosis

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ABSTRACT

Background: Cortical reorganization and plasticity may compensate for structural damage in Multiple Sclerosis (MS). It is important to establish sensitive methods to measure these compensatory mechanisms, as they may be of prognostic value.
Objective: To investigate the association between the degree of cortical plasticity and cognitive performance and to compare plasticity between MS patients and healthy controls (HCs).
Methods: The amplitudes of the motor evoked potential (MEP) pre and post quadripulse stimulation (QPS) applied over the contralateral motor cortex served as measure of the degree of cortical plasticity in 63 patients with relapsing-remitting MS (RRMS) and 55 matched HCs. The main outcomes were the correlation coefficients between the difference of MEP amplitudes post and pre QPS and the Symbol Digit Modalities Test (SDMT) and Brief Visuospatial Memory Test-Revised (BVMT-R), and the QPS^{x}group interaction in a mixed model predicting the MEP amplitude.
Results: SDMT and BVMT-R correlated significantly with QPS-induced cortical plasticity in RRMS patients. Plasticity was significantly reduced in patients with cognitive impairment compared to patients with preserved cognitive function and the degree of plasticity differentiated between both patient groups. Interestingly, the overall RRMS patient cohort did not show reduced plasticity compared to HCs.
Conclusions: We provide first evidence that QPS-induced plasticity may inform about the global synaptic plasticity in RRMS which correlates with cognitive performance as well as clinical disability. Larger longitudinal studies on patients with MS are needed to investigate the relevance and prognostic value of this measure for disease progression and recovery.

1. Introduction

Multiple Sclerosis (MS) is a chronic disease of the central nervous system, characterized by inflammatory, demyelinating lesions in the brain and spinal cord [1,2]. In approximately 80% of patients, the disease initiates as relapsing-remitting MS (RRMS) with episodes of sub-acutely developing clinical symptoms and neurological deterioration with successive recovery [3]. In patients with primary or secondary progressive MS, symptoms evolve in the absence of relapses [4], are less responsive to immunomodulatory therapy [5] and believed to result from at least partially distinct pathophysiological mechanisms [4].

MS symptoms can affect almost any function of the central nervous system. Approximately 85% of MS patients report

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spasticity, paresis, and disturbances of sensibility at some point during the disease [6,7]. MS also often results in neuropsychiatric and cognitive symptoms including depression, fatigue and cognitive impairment (CI) [2]. CI affects approximately 40–65% of MS patients [8,9], often occurs early on in the disease [10] and significantly affects the patients' quality of life, vocational status and social activities [11,12].

Remarkable advances have been made in the diagnosis and treatment of MS. This includes progress in research on the clinico-radiological paradox [13,14], referring to the at times staggering discrepancy between radiological parameters and clinical outcomes. Yet, prediction of individual clinical course remains impossible. suggesting that accumulation of cerebral lesions and atrophy are not the only determinants of disability in MS.

Compensatory mechanisms of cortical reorganization and plasticity may be an important additional factor, as they can offset deficits caused by demyelination and neurodegeneration [15,16]. If these compensatory reserve mechanisms are exhausted, structural damage may directly translate into disability. This is particularly relevant for cognitive decline, as it often results from complex pathologies, involving both white and gray matter [17]. Therefore, the development of reliable methods to assess cortical plasticity and the compensatory reserve are of paramount interest as they could be of prognostic value.

Repetitive transcranial magnetic stimulation (rTMS) of the cortex may ideally be suited for this undertaking. Transcranial magnetic stimulation (TMS) is a non-invasive method to stimulate the cortex by inducing an electrical current. Using repetitive stimulation, the cortical excitability can be changed by modulating mechanisms of synaptic plasticity. Both facilitation, comparable with long term potentiation (LTP), and inhibition, comparable with long term depression, can be induced by different stimulation frequencies [18,19].

Previous studies on rTMS-induced plasticity of the motor cortex in RRMS patients revealed conflicting results regarding differences compared to healthy controls (HCs) during remission [20–24]. During relapse, preserved plasticity was associated with better functional recovery [25] and a reversal of the direction of induced plasticity may reflect compensatory metaplastic effects on the cortical level [26].

These results suggest that rTMS is an appropriate technique to measure the compensatory reserve in MS patients with possibly high relevance for individual prognosis. However, a limiting factor is that conventional rTMS plasticity protocols show high variability and up to 60% non-responder rates [27]. The aim of the present study was to assess motor cortex plasticity in RRMS patients and HCs using quadripulse stimulation (QPS), supposedly one of the most effective plasticity inducing protocols with lowest variability [28–30]. QPS has been shown to selectively modulate the excitatory glutamatergic cortical neuronal network, whereas other TMS protocols also modulate inhibitory GABA-ergic networks [31,32]. Since the glutamatergic network presumably plays an important role in the pathophysiology of MS [33,34], QPS may represent a more reliable method to measure cortical plasticity in MS than other TMS protocols.

Using this new approach, we investigated the relationship between cortical plasticity and two of the most frequently affected cognitive domains, namely information processing speed (IPS) and visuospatial short-term memory and learning [2,35,36]. Research on the relationship between TMS-induced plasticity and cognitive performance using other TMS protocols indicates that reduced plasticity may be associated with cognitive deficits [37,38]. We therefore aimed to replicate these findings with our TMS protocol using the correlation coefficients between QPS-induced plasticity and our cognitive outcome measures as the primary outcomes.

We further aimed at comparing RRMS patients with HCs regarding the degree of QPS-induced plasticity to resolve the ambiguous current research status. We expected to find reduced plasticity in RRMS patients, indicated by a significant QPS^{x}group interaction in a linear mixed model.

2. Materials and methods

2.1. Subjects

Patients diagnosed with definite RRMS according to the revised McDonald criteria [39] and age-, sex- and education-matched HCs were recruited between May 2018 and May 2021 at the University Hospital in Düsseldorf, Germany. The following exclusion criteria were applied: (1) history of diseases of the central or peripheral nervous system other than RRMS, (2) history of psychiatric diseases potentially affecting cognition other than remitted depressive episodes, (3) presence of any contraindication for TMS, (4) drug or alcohol abuse. Exclusion criteria were incorporated in a standardized questionnaire, including a TMS safety screening [40]. Patients had to be relapse-free for at least 30 days and sufficient visual acuity to recognize visual material in the neuropsychological assessment was required for all subjects. Informed written consent was provided prior to participation. The study was approved by the ethical committee of the medical faculty of the Heinrich-Heine-University Düsseldorf (study-number 2018–16) and carried out in accordance with the declaration of Helsinki.

3. Experimental design

Data were assessed in a single session using a standardized protocol: (1) neurological and neuropsychological examination, (2) cortical plasticity measurements using TMS.

3.1. Neurological and neuropsychological assessment

Measures of IPS, visuospatial short-term memory and learning, depression, anxiety, and fatigue were applied by trained personnel experienced in the treatment of patients with MS. The Expanded Disability Status Scale (EDSS) [41] and medical history were determined by experienced neurologists. The EDSS is a widely accepted method to quantify MS-related disability on an ordinal scale ranging from 0 (no neurological signs) to 10 (death due to MS).

To assess IPS and visuospatial short-term memory and learning, the oral version of the Symbol Digit Modalities Test (SDMT) [42] and three learning trials of the Brief Visuospatial Memory Test Revised (BVMT-R) [43] were used, respectively. To identify patients with CI, SDMT and BVMT-R z-scores were calculated based on the norms provided in the German SDMT validation study [44] and BVMT-R manual [43]. In line with the defined and utilized cut-off value for the SDMT in Germany [44] and to ensure comparability between tests, patients with z-scores lower than −1.68 in either of these two tests were classified as cognitively impaired.

Depression, anxiety and fatigue were measured with the total subscale scores of the Hospital Anxiety and Depression Scale [45] and the total score of the Fatigue Scale for Motor and Cognitive Functions [46] representing a measure of trait fatigue, respectively, to control for potential confounders.

3.2. Cortical plasticity measurements using TMS

Participants were seated in a comfortable reclining chair with arms placed on cushioned armrests. The motor evoked potential (MEP) amplitude of the right first dorsal interosseous (FDI) muscle

served as measure for cortical excitability. First, baseline MEP were recorded, followed by QPS, which was used as a plasticity inducing rTMS protocol. After the intervention, MEPs were recorded every 10 min during a follow up of 60 min (Figure A1, *Supplement*). The degree of MEP amplitude changes induced by QPS served as measure of cortical plasticity.

3.2.1. Transcranial magnetic stimulation

Single pulse monophasic TMS was applied to the left primary motor cortex using a hand-held figure-of-eight coil (70 mm outer diameter, The Magstim Company Ltd., Whitland, UK) connected to a Magstim BiStim [2] (The Magstim Company Ltd., Whitland, UK) stimulator. The coil was positioned tangentially to the skull with the handle pointing posterolateral at an angle of 45° to the sagittal plane to ensure a posterior-anterior current direction in the brain. The FDI hotspot was defined as the optimal position for eliciting the largest MEP in the target muscle. Starting 5 cm lateral and 1 cm ventral to the vertex, we approximated this site before each experiment in 1 cm steps until reliable MEPs were evoked in the FDI. Subjects were told to keep the target muscle relaxed, to minimize verbal interactions with the experimenter and to keep count of the number of applied stimuli during the session to avoid MEP changes due to muscle innervation or shifts in attention. The selected FDI hotspot was marked on the subjects' head using a colorful pen to ensure consistent coil position across stimulations.

We applied single pulses of TMS to define motor thresholds. Resting motor threshold was determined as the minimum stimulus intensity producing ≥50 μV MEPs in at least five out of ten trials at rest using the relative frequency method [47]. Accordingly, active motor threshold was determined as the minimum stimulus intensity able to produce ≥100 μV MEPs in at least five out of ten consecutive trials during 10–20% of maximal FDI muscle contraction. MEP latency was measured by applying ten single pulses with an intensity of 140% of the resting motor threshold while the subject maintained a contraction of ~30% of the maximum voluntary activity at the target muscle, as assessed by surface electromyography (EMG) and monitored in real time on an oscilloscope (DS1074B, Batronix Rigol, Preetz, Germany). For statistical analyses, the mean latency of the ten trials was calculated [48].

3.2.2. Motor evoked potential recordings

MEPs were recorded by surface EMG using Ag–AgCl-electrodes (20 × 15 mm, Ambu, Ballerup, Denmark) in a belly-tendon montage. The signal was amplified (Digitimer D360, Digitimer

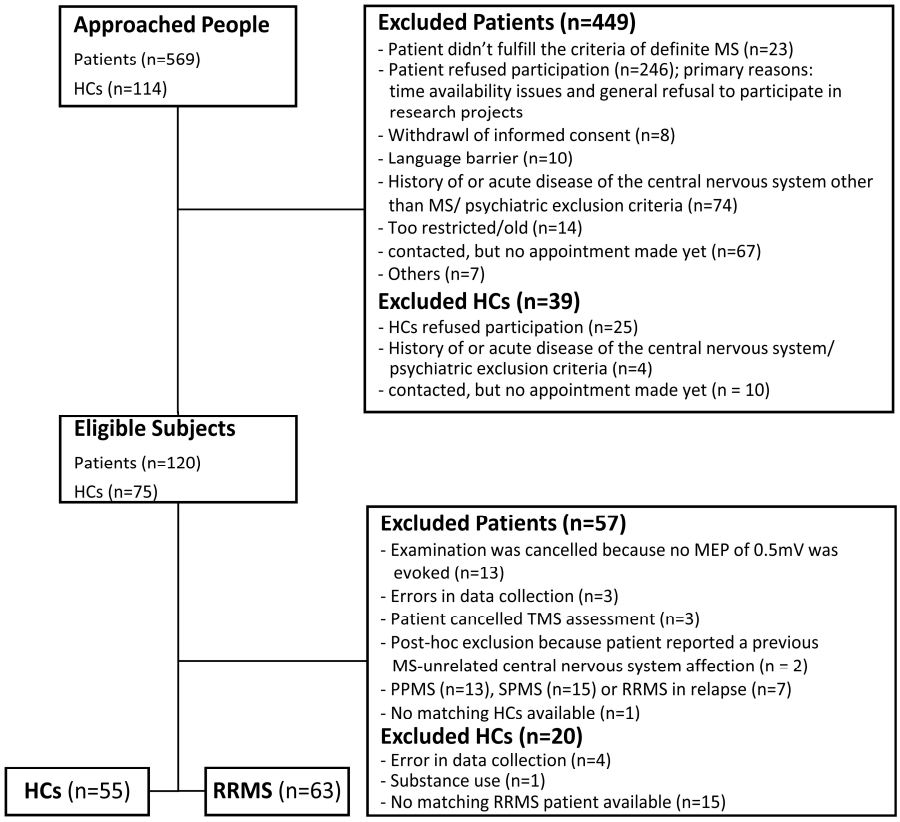

Fig. 1. Flowchart of the enrollment of subjects The flowchart presents the numbers of subjects at each step of the study. HCs=Healthy controls; RRMS = Relapsing-remitting Multiple Sclerosis; PPMS; Primary-progressive Multiple Sclerosis; SPMS= Secondary-progressive Multiple Sclerosis.

Ltd, Hertfordshire, UK, frequency band of the filter: 100–5000 Hz), digitized at a sampling rate of 5 kHz and stored on a computer for offline analysis (Signal version 6.02, Cambridge Electronic Design Ltd., Cambridge, UK). MEP responses evoked by single pulse TMS, adjusted to be ~0.5 mV, were recorded pre QPS. For each of the post interventional time points, MEP responses evoked by the same stimulation intensity, were recorded. At each time point 12 MEPs were averaged. Trials contaminated with voluntary muscle activity and/or artefacts impeding the assessment's interpretation were discarded from analyses, resulting in an average of 11 utilized MEPs for each time point and subject.

3.2.3. Quadripulse stimulation

We used the QPS protocol originally described by Hamada et al. [31], supposedly leading to a more homogenous and efficient stimulation of neuron populations than biphasic rTMS. Four stimulators (Magstim 200 [2], The Magstim Company Ltd., Whitland, UK) were connected using a combining module (The Magstim Company Ltd., Whitland, UK) to allow for monophasic rTMS. 360 TMS-bursts, each consisting of four monophasic TMS-pulses with an interstimulus-interval of 5 ms, were repeatedly applied at a frequency of 0.2 Hz to induce LTP-like plasticity. The stimulation intensity was set at 90% of the active motor threshold and the subject was told to keep the target muscle relaxed, which was monitored using an oscilloscope.

3.2.4. Statistical analyses

Since this is the first study using QPS in a cognition study with MS patients, we could not rely on previous data to calculate sample size. It was therefore based on the number of patients and matched HCs eligible for this study.

According to the nature of the data, clinical and demographic group differences between patients and HCs were assessed using Fisher's exact test for categorical data and Mann-Whitney-U-test for continuous variables, since requirements of parametric testing were not met in at least one group. MEP amplitude changes after QPS were used as an operationalisation of plasticity as they can be investigated with high reproducibility and standardization [31].

To investigate the association between QPS-induced LTP and cognitive performance, the difference between the maximum of the six mean post MEPs and the pre MEP amplitude (ΔMEP) was calculated, reflecting the maximum degree of cortico-spinal excitability change following QPS. Spearman's Rank correlation coefficients of ΔMEP with SDMT and BVMT-R total scores were computed and Bonferroni corrected *p*-values below .05 were considered statistically significant.

Post-hoc, Spearman's Rank correlation coefficients of ΔMEP with age, education, MEP latency, EDSS, depression, anxiety, and fatigue were calculated as these factors could impact TMS-induced plasticity. Due to the exploratory nature of these post-hoc analyses, no multiple comparisons correction was applied. All above analyses were conducted using IBM SPSS Statistics (version 25).

To control for potentially confounding factors on MEP responses and cognition, stepwise linear regression models predicting the performance on SDMT and BVMT-R in patients based on ΔMEP and the before mentioned covariates were conducted. Since there is evidence of differences in cognitive performance between the biological sexes [49] and sex-specific disruption of cortical mechanisms in MS [50], the biological sex was added as a binary covariate as well. Continuous variables were centered at the sample mean and analysis was carried out using the MASS package in R Studio (version 1.3.1093). Listwise deletion was applied in case of missing data.

Group comparisons between patients and HCs regarding QPS-induced motor plasticity were carried out with linear mixed-effects models using the nlme package in R Studio (version 1.3.1093) to account for clustering of pre and post assessment within subjects. For each subject, the pre QPS MEP, controlled to be ~0.5 mV, and the maximum of the six mean post MEPs entered analyses to compare the maximum degree of cortico-spinal excitability change after QPS between patients and HCs. Details on model computation are provided in Methods A1, *Supplement*.

Post-hoc, clinical and demographic group differences between patients with and without CI were assessed using the same testing procedures as described for the group comparison of RRMS patients and HCs. Additionally, receiver-operating characteristic analysis was conducted with IBM SPSS Statistics (version 25) to evaluate the ability of ΔMEP to discriminate between patients with and without CI. The area under the curve (AUC) was calculated and transformed into Cohen's d according to Rice and Harris [51].

4. Data availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

5. Results

5.1. Neurological and neuropsychological sample characteristics

Out of 683 approached people, 63 patients with RRMS and 55 age-, sex- and education matched HCs were included in the study (Fig. 1). Descriptive statistics are presented in Table 1. Proving successful matching, no significant differences between RRMS patients and HCs regarding age, gender or education were found. TMS thresholds were comparable in both groups. However, MEP latency was significantly longer in the RRMS sample and significantly more patients than HCs presented with clinical anxiety and depression scores. It should be noted that these scores do not reflect psychiatric diagnoses, but only indicate the presence of symptoms during the past week. In line with this and our exclusion criteria, all patients denied ongoing depressive episodes or anxiety disorders. The neuropsychological tests revealed significantly worse performance in IPS and visuospatial short-term memory and learning for RRMS patients.

5.2. Correlations of neuropsychological performance with QPS-induced neural plasticity

Correlational analyses of SDMT and BVMT-R total scores with QPS-induced plasticity revealed significant positive correlations between these performance measures and ΔMEP in RRMS patients (SDMT: $r_s = 0.45$, Bonferroni-corrected $p < .001$; BVMT-R: $r_s = 0.40$, Bonferroni-corrected $p = .002$). As presented in Fig. 2, better performances in both IPS and visuospatial short-term memory and learning were associated with higher ΔMEP. In HCs, however, there was no significant correlation with ΔMEP (SDMT: $r_s = 0.23$, Bonferroni-corrected $p = .19$; BVMT-R: $r_s = -0.11$, Bonferroni-corrected $p = .86$).

Post-hoc, no association between depression, anxiety and fatigue with ΔMEP was found in either of the two groups (Table 2). There were, however, significant negative correlations with MEP latency ($r_s = -0.31$, $p = .02$), age ($r_s = -0.25$, $p = .045$), and EDSS ($r_s = -0.26$, $p = .04$) in RRMS patients. Independent of these confounding factors, stepwise linear regression modeling revealed a significant influence of ΔMEP on both BVMT-R ($\beta = 1.20$, $p = .03$) and SDMT ($\beta = 2.83$, $p = .006$) (Table 3).

Table 1
Participant characteristics.

Characteristic	RRMS ($N = 63$)	HCs ($N = 55$)	p-value
Sex, No. (%), female	42 (67)	36 (66)	>.99
Handedness, No. (%), right	56 (89)	49 (89)	>.99
Age, median (min-max), years	39 (20–61)	33 (21–67)	.60
Education, median (min-max), years	16 (8–22)	16 (12–21)	.12
AMT, median (min-max), % MSO	38 (27–73)	39 (24–48)	.16
RMT, median (min-max), % MSO	48 (33–81)	48 (31–63)	.39
MEP 0.5 mV, median (min-max), % MSO	59 (36–100)	57 (35–88)	.06
MEP latency, median (min-max), ms[a]	23.05 (17.74–37.83)	21.77 (18.88–27.18)	**.01**
ΔPost-Pre MEP amplitude, median (min-max), mV	0.48 (−0.30-3.17)	0.56 (−0.01-2.68)	.56
BVMT-R			
Total learning score, median (min-max)	25 (2–35)	29 (8–36)	**<.001**
z-score, median (min-max)[b]	−0.29 (−4.70-1.92)	0.80 (−3.53-1.82)	**<.001**
SDMT			
correct items, median (min-max)	55 (19–84)	63 (33–98)	**<.001**
z-score, median (min-max)[c]	−0.48 (−4.12- 2.14)	0.43 (−2.17 - 3.67)	**<.001**
HADS, No. (%), clinical[d]			
Anxiety	9 (14)	0 (0)	**.004**
Depression	9 (14)	0 (0)	**.004**
Disease duration, median (min-max), years	9.35 (0–30)		
EDSS, median (min-max)	1.5 (0–7.5)		
FSMC, No. (%), mild/moderate/severe[e]			
Motor	9 (14)/8 (13)/27 (43)		
Cognitive	8 (13)/11 (18)/21 (33)		
DMT exposure, No. (%)			
None	11 (18)		
Natalizumab	21 (33)		
Ocrelizumab	19 (30)		
Glatiramer acetate	2 (3)		
Dimethyl fumarate	2 (3)		
Interferon beta-1a	2 (3)		
Fingolimod	2 (3)		
Cladribine	2 (3)		
Alemtuzumab	1 (2)		
Teriflunomid	1 (2)		

Note. p-values < .05 are in boldface and based on two-tailed analysis. RRMS = Relapsing-remitting Multiple Sclerosis. HCs= Healthy controls. AMT = Active Motor Threshold. RMT = Resting Motor Threshold. MEP = Motor evoked potential. MEP 0.5 mV = Stimulation intensity producing a reliable MEP of ~0.5 mV. MSO = Maximal stimulator output. BVMT-R = Brief Visuospatial Memory Test Revised. SDMT= Symbol Digit Modalities Test. HADS=Hospital Anxiety and Depression Scale. EDSS = Expanded Disability Status Scale. FSMC= Fatigue Scale of Motor and Cognition. Classification based on cut-off scores defined in the manual. DMT = Disease-modifying therapy.
[a] Missings as follow: 6 RRMS, 15 HCs.
[b] Calculation based on the BVMT-R manual [1].
[c] Calculation based on German norms [2].
[d] Missings as follow: 2 HCs. Classification as clinical based on scores ≥11.
[e] Classification as mild, moderate, and severe based on cut-offs provided in the FSMC. [3].

5.3. Differences in QPS-induced plasticity between patients with Multiple Sclerosis and HCs

Fig. 3a illustrates the averaged ΔMEP per time point for HCs and RRMS patients. In both groups, ΔMEP strongly increased post QPS intervention and LTP-like effects lasted until the end of the experiment, suggesting equal degrees of QPS-induced plasticity in RRMS patients and HCs. To test group differences for statistical significance, linear mixed-effects models were carried out as described above.

The model including QPS, group, age and QPS^{x}group was superior compared to all other models and revealed significant effects of the QPS intervention ($\beta = 0.63$, $p < .001$) and age ($\beta = -0.03$, $p = .03$) (Table 4). Prior to stimulation, MEP amplitudes were equal in both groups ($\beta - -0.01$, $p - .80$) since they were experimentally adjusted to be ~0.5 mV in all subjects. Overall, the final model fit was satisfying with a conditional R^2 of 0.97 and a marginal R^2 of 0.37. Including depression, anxiety, latency and their interaction with the intervention did not improve model fit and none of these predictors reached statistical significance. There further was no significant interaction of age^{x}group or age^{x}QPS in rejected models.

For clarity, we plotted estimated MEP amplitudes pre and post QPS for a hypothetical HC and RRMS patient, representative of the average subject in our study, based on the fixed effects of the model (Fig. 3b). This illustrates, on average, an equally strong increase of the MEP amplitude post QPS in both groups. Yet, there is considerable variation between subjects as indicated by an adjusted intraclass correlation coefficient of 0.95.

5.4. Differences in QPS-induced plasticity between cognitively impaired and unimpaired patients

Due to the significant correlation between QPS-induced plasticity and SDMT and BVMT-R total scores, we compared the degree of QPS-induced plasticity between patients with and without CI. Characteristics of the two groups are provided in Table A1, *Supplement*.

Fig. 3c illustrates the averaged ΔMEP per time point for patients with 1) no CI and 2) impairment in at least one of the two tests. In patients without CI, ΔMEP continuously increases post QPS intervention, indicating strong LTP-like effects. In patients with CI, ΔMEP peaks 40 min post QPS intervention and is lower than in patients without CI across all time points, indicating reduced QPS-induced plasticity in patients with CI. This is also true when investigating ΔMEP per time point for patients with impairment in the SDMT and BVMT-R separately (Figure A2, *Supplement*).

As described above, group differences were tested for statistical significance using linear mixed-effects models. The model

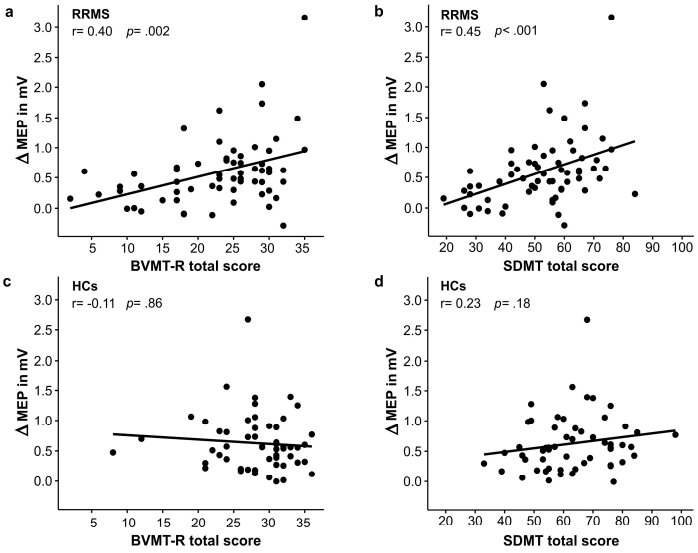

Fig. 2. Correlations of the difference between pre and post QPS MEP amplitude with SDMT and BVMT-R in patients with RRMS (a,b) and HCs (c,d) This figure shows the correlations of the difference between the pre and post QPS MEP amplitude with the SDMT as a measure of information processing speed and the BVMT-R as a measure of visuospatial short-term memory and learning separately for patients with RRMS and HCs. HCs=Healthy Controls; RRMS = Relapsing-remitting Multiple Sclerosis; BVMT-R = Brief Visuospatial Memory Test Revised; SDMT= Symbol Digit Modalities Test; QPS = Quadripulse stimulation; MEP = Motor evoked potential; ΔMEP = Difference between the maximum of the six mean MEP amplitude after stimulation and the MEP amplitude before stimulation.

including QPS, group, fatigue, and QPS^{x}group was superior compared to all other models and revealed significant effects of QPS intervention ($\beta = 0.69$, $p < .001$), fatigue ($\beta = -0.04$, $p = .03$), and QPS^{x}group ($\beta = -0.31$, $p = .04$) (Table A2, *Supplement*). The final model achieved a satisfying fit with a conditional and marginal R^2 of 0.95 and 0.38, respectively.

Table 2
Correlation coefficients of clinical characteristics with Δ MEP.

	RRMS ($N = 63$)		HCs ($N = 55$)	
	r_s	*p-value*	r_s	*p-value*
BVMT-R	0.40	**.002**	−0.11	.86
SDMT	0.45	**<.001**	0.23	.19
Post Hoc Analyses				
MEP Latency[a]	−0.31	**.02**	−0.10	.55
Age	−0.25	**.045**	0.01	.96
Education	0.19	0.26	0.08	>.99
HADS			−0.16 -0.23	.26 .09
Depression	−0.08	.54		
Anxiety	0.19	.14		
FSMC				
Motor	−0.20	.12		
Cognitive	−0.18	.16		
EDSS[b]	−0.26	**.04**		

Note. *p*-values <.05 are in boldface and based on two-tailed analysis. *p*-values of the BVMT-R and SDMT were Bonferroni-corrected for multiple testing (two tests). ΔMEP = difference between the maximum of the six mean MEP amplitude after stimulation and the MEP amplitude before stimulation. RRMS = Relapsing-remitting Multiple Sclerosis. HCs= Healthy controls. BVMT-R = Brief Visuospatial Memory Test-Revised. SDMT= Symbol Digit Modalities Test. MEP = Motor evoked potential. HADS= Hospital Anxiety and Depression Scale. FSMC = Fatigue Scale of Motor and Cognitive Function. EDSS = Expanded Disability Status Scale.

[a] Missing as follows: 6 RRMS, 15 HCs.

[b] One RRMS patient excluded from analysis because EDSS did not accurately reflect the patient's disability. According to the examining neurologist the patient showed clear signs of aggravation in the examination of motor symptoms, potentially due to an overlying somatoform disorder.

The model-estimated pre and post QPS MEP amplitudes based on the fixed effects for a representative patient with and without CI are presented in Fig. 3d. An increase of the MEP amplitude post QPS was observed in both groups. However, it was significantly stronger in patients without CI than in patients with CI. Again, we found considerable variation between subjects with an intraclass correlation coefficient of 0.93.

The receiver-operating characteristic analysis revealed moderate accuracy (*AUC* = 0.69; *d* = 0.68) of ΔMEP to differentiate

Table 3
Multivariable linear regression model of BVMT-R and SDMT total score in RRMS.

	β-coefficient (95% CI)	SE_b	t-value	p
BVMT-R				
Intercept	+21.59 (+18.83; +24.36)	1.37	15.68	**<.001**
ΔMEP	+1.20 (+0.13; +2.27)	0.53	2.25	**.03**
MEP Latency	−1.54 (−2.77; −0.30)	0.61	−2.50	**.02**
Age	−2.52 (−4.03; −1.02)	0.75	−3.37	**.001**
Education	+3.32 (+1.60; +5.04)	0.86	3.87	**<.001**
Fatigue	+1.79 (+0.10; +3.48)	0.84	2.13	0.04
Sex[a]	+2.77 (−0.50; +6.00)	1.61	1.72	0.09
SDMT				
Intercept	+53.88 (+51.01; +56.75)	1.43	37.67	**<.001**
ΔMEP	+2.83 (+0.82; +4.84)	1.00	2.82	**.007**
Age	−4.85 (−7.60; −2.10)	1.37	−3.54	**<.001**
MEP Latency	−3.92 (−6.78; −1.06)	1.43	−2.75	**.008**
Education	+3.23 (+0.70; +5.76)	1.26	2.56	**.01**

Note. Two-tailed *p*-values and CI are displayed. *p*-values <.05 are in boldface. MEP = Motor evoked potential. BVMT-R = Brief Visuospatial Memory Test-Revised. SDMT= Symbol Digit Modalities Test. ΔMEP = difference between the maximum of the six mean MEP amplitude after stimulation and the MEP amplitude before stimulation. All SE are robust SE based on HC4-method and *t*-and *p*-values were derived from robust SE.
Adjusted $R^2_{BVMT-R} = 0.38$ ($p < .001$). Adjusted $R^2_{SDMT} = 0.37$ ($p < .001$).

[a] Male = 0, female = 1.

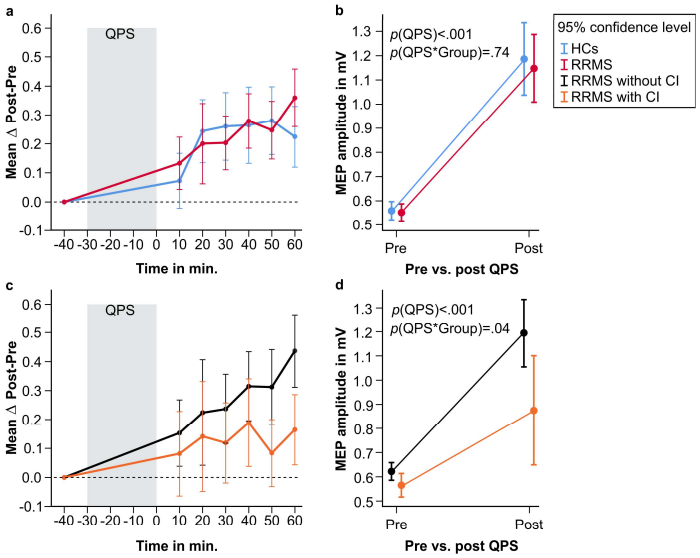

Fig. 3. QPS-induced plasticity in patients with RRMS compared to matched HCs (a,b) and in RRMS patients with cognitive impairment compared to patients without cognitive impairment (c,d). (b) and (d) show the predicted MEP amplitude based on the fixed effects of the linear mixed models. This figure shows the level of QPS-induced plasticity in different clinical subgroups. The upper part of the figure (a,b) displays QPS-induced plasticity in patients with RRMS compared to matched HCs. The lower part of the figure (c,d) shows QPS-induced plasticity in RRMS patients with and without cognitive impairment. The left part of the figure (a,c) shows the averaged difference between the pre and post QPS MEP amplitude per time point. The right part of the figure (b,d) shows the predicted MEP amplitude based on the fixed effects of the linear mixed models. QPS = Quadripulse stimulation; MEP = Motor evoked potential; HCs=Healthy Controls; RRMS = Relapsing-remitting Multiple Sclerosis; CI=Cognitive impairment.

between both patient groups and high accuracy when investigating concurrent impairment in both SDMT and BVMT (*AUC* = 0.83; *d* = 1.33) and impairment in the SDMT (*AUC* = 0.75; *d* = 0.95) and BVMT (*AUC* = 0.72; *d* = 0.81) separately (Fig. 4).

6. Discussion

We present the first study investigating QPS of the motor cortex as a measure of global cortical plasticity in MS, a method that has previously demonstrated a higher reproducibility than other rTMS paradigms [28]. Using this technique we identified significant correlations of QPS-induced plasticity with the SDMT and BVMT-R in a large patient cohort. Measures of IPS and visuospatial short-term memory and learning assess cognitive core domains which are of high relevance for daily living of patients [11].

The fact that this association remained significant when controlling for confounding factors such as the MEP latency, supposedly at least partly representing the integrity of the pyramidal tract, and that we also found a negative association of QPS-induced plasticity with the EDSS, highlights the relevance of global synaptic plasticity for the clinical status of patients. The clinical relevance of this method is further supported by the fact that QPS-induced plasticity was significantly lower in cognitively impaired compared to cognitively preserved patients and by our finding of no correlation in HCs. These results suggest that mechanisms of reserve only become relevant when a sufficient degree of pathology, that needs to be compensated, is present [52,53]. In line with

Table 4
Multivariable linear mixed-effect model of MEP amplitude in HCs and RRMS patients before and after QPS.

Fixed Effects					Random Effects
	β-coefficient (95% CI)	*SE$_b$*	*t-value*	*p*	*s* [2]
Intercept	+0.56 (0.52; 0.60)[a]	0.02	28.65	**<.001**	
Pre QPS	Reference				
Post QPS	+0.63 (+0.49; +0.77)[a]	0.07	8.88	**<.001**	
HCs	Reference				
MS	−0.01 (−0.06; +0.05)	0.03	−0.26	.80	
Age	−0.03 (−0.05; −0.003)[a]	0.01	−2.20	**.03**	
Post QPS*MS	−0.03 (−0.22; −0.16)	0.10	−0.33	.74	
Subject*Pre QPS					0.01
Subject*Post QPS					0.31
Residual					0.09

Note. Two-tailed 95% CI and *p*-values are displayed. *p*-values <.05 are in boldface. QPS = Quadripulse stimulation. HCs=Healthy controls. MS = Multiple Sclerosis. RRMS = Relapsing-remitting Multiple Sclerosis. MEP = Motor evoked potential. R^2(conditional) = 0.97. R^2(marginal) = 0.37. Adjusted Intraclass Correlation Coefficient = 0.95.
[a] Indicates statistical significance. *t*- and *p*-values are based on asymptotic Wald test.

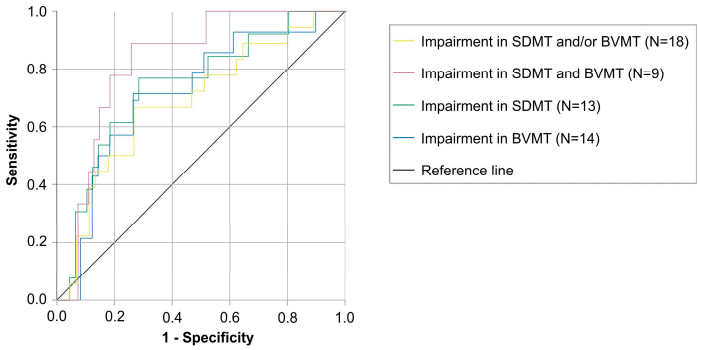

Fig. 4. Receiver-operating characteristic curve illustrating the accuracy of ΔMEP to differentiate between patients with and without cognitive impairment. This figure illustrates the receiver-operating characteristic curve of the accuracy of the difference between the maximum of the six mean post MEPs and the pre MEP amplitude to differentiate between patients with and without cognitive impairment. BVMT-R = Brief Visuospatial Memory Test Revised; SDMT= Symbol Digit Modalities Test; ΔMEP = Difference between the maximum of the six mean MEP amplitude after stimulation and the MEP amplitude before stimulation.

this, we revealed that the degree of plasticity changes can accurately discriminate between patients with and without impairment in the SDMT and BVMT-R. This association seems to be rather unaffected by levels of fatigue, as different degrees of plasticity in patients with and without CI occurred despite similar fatigue scores. Furthermore, there was no significant difference between our patient cohort and HCs, even though more than half of our patients suffered from at least moderate fatigue.

To investigate a possible influence of cortical plasticity on disease progression in more depth, longitudinal studies are needed, which are already underway.

Importantly, we found that the degree of cortical plasticity was not generally reduced in this overall mildly affected group of patients compared to HCs. Together with our finding of reduced plasticity in CI patients, this implies that promotion of synaptic plasticity may be a promising tool to prevent clinical deterioration and CI specifically. Further, promotion of synaptic plasticity could be used as a rehabilitation effort.

Interestingly, the degree of cortical plasticity was negatively associated with disease severity in terms of EDSS. Thus, our findings may help to integrate the conflicting previous results, potentially arising from more severely affected patients in the cohorts with reduced TMS-induced plasticity[22,23] than in those with preserved plasticity.[20,21] However, LTP-like plasticity can also be altered in patients with low disability and short disease duration [24]. To further explore a potential association between disease severity and synaptic plasticity, future research should report detailed clinical characteristics of patients and investigate subgroups with different disease severities.

This study provides several strengths. Firstly, we investigated only RRMS patients and matched HCs, reducing the risk of artefacts linked to disease subtype. Further, we report the largest ever reported sample size in rTMS research in patients with MS, which improves reliability of the results. Lastly, we used a TMS protocol that may be more sensitive and reliable for the functional measurement of cortical plasticity than previously used TMS paradigms [29,30,32] with higher response variability and non-responder rates of up to 60% [27]. We believe that focusing uniquely on RRMS with various degrees of disease severity constitutes the most rigorous approach to investigate the interplay of cortical plasticity and autoimmune pathology that is accessible to standard immunomodulatory therapy. Further and larger studies are warranted to compare these findings to matching cohorts of the less frequent progressive MS subtypes, where neurodegeneration, cortical pathology, and the innate immune system are thought to be more relevant.

Limitations are the cross-sectional design, unequal group sizes, lack of physical disability readouts besides EDSS, and lack of imaging data. Our work primarily focused on excitatory circuits because MS has been associated with alterations in the glutamatergic network [33,34] and since QPS has been shown to specifically modulate excitatory circuits and to leave inhibitory circuits unchanged [31]. However, inhibitory circuits can be altered in MS as well [54]. We therefore encourage future research to explore the interplay of excitatory and inhibitory mechanisms. Moreover, the impact of MS pathology on synaptic plasticity should be further investigated, e.g. by integrating advanced MRI-imaging techniques. Lastly, we focused on the left hemisphere only to keep the examination time reasonable, but encourage future research to also explore hemispheric differences and effects of handedness.

Our findings are novel and of great importance as they suggest that QPS can inform about the degree of synaptic plasticity well beyond the motor cortex, which is consistent with previous reports in HCs [55–59]. Other TMS-protocols have already been used to study the prognostic value of cortical plasticity regarding recovery after relapse [25] and clinical progression [60]. We suggest to also apply QPS in prospective studies to investigate a potential prognostic value of QPS-induced plasticity for relapse recovery and long-term disability progression.

Furthermore, longitudinal studies are warranted to investigate the influence of synaptic plasticity on CI in more detail. TMS-induced plasticity may not only be related to compensatory but also to pathogenic mechanisms like neurodegeneration and inflammation. In fact, reduced synaptic plasticity itself may lead to cognitive deficits and neuronal network dysfunctions and could therefore not only play a role as a mediator but also as a cause of cognitive decline [17].

In conclusion, we provide first evidence that QPS-induced plasticity may inform about the global synaptic plasticity in RRMS which correlates with cognitive performance as well as clinical disability. Larger longitudinal studies on patients with MS are needed to investigate the relevance and prognostic value of this measure for disease progression and recovery.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: C. Balloff reports no disclosures relevant to the manuscript. I.-K. Penner received honoraria for speaking at scientific meetings, serving at scientific advisory boards and consulting activities from Adamas Pharma, Almirall, Bayer Pharma, Biogen, BMS, Celgene, Desitin, Sanofi-Genzyme, Janssen, Merck, Novartis, Roche, and Teva. She received research support from the German MS Society, Celgene, Novartis, Roche, and Teva. M. Ma reports no disclosures relevant to the manuscript.I. Georgiades reports no disclosures relevant to the manuscript.L. Scala reports no disclosures relevant to the manuscript.N. Troullinakis reports no disclosures relevant to the manuscript.J. Graf received travel/meeting/accommodation reimbursements from Biogen, Merck Serono, Sanofi-Genzyme, Grifols, and a Research Fellowship from the Deutsche Forschungsgemeinschaft (project number 438899010).D. Kremer received travel grants from GeNeuro and Merck, refund of congress participation fees from GeNeuro, Merck, and Servier, consulting fees from Grifols, payment for lectures from Grifols, and support for research projects from Teva and was funded by the Deutsche Forschungsgemeinschaft (DFG).O. Aktas reports grants from the German Science Foundation (DFG) and the German Ministry of Education and Research (BMBF), outside of the submitted work, and personal fees from Alexion, Bayer HealthCare, Biogen, Merck Serono, Novartis, Roche, Teva, and MedImmune, outside of the submitted work.H.-P. Hartung has received fees for serving on steering and data monitoring committees from Bayer Healthcare, Biogen, Celgene BMS, CSL Behring, GeNeuro, MedImmune, Merck, Novartis, Octapharma, Roche, Sanofi Genzyme, TG Therapeutic sand Viela Bio; fees for serving on advisory boards from Biogen, Sanofi Genzyme, Merck, Novartis, Octapharma, and Roche; and lecture fees from Biogen, Celgene BMS, Merck, Novartis, Roche, Sanofi Genzyme.S.G. Meuth has received honoraria for lecturing and travel expenses for attending meetings from Almirall, Amicus Therapeutics Germany, Bayer Health Care, Biogen, Celgene, Diamed, Genzyme, MedDay Pharmaceuticals, Merck Serono, Novartis, Novo Nordisk, ONO Pharma, Roche, SanofiAventis, Chugai Pharma, QuintilesIMS and Teva, and research funding from the German Ministry for Education and Research (BMBF), the Deutsche Forschungsgemeinschaft, the Else Kröner Fresenius Foundation, the German Academic Exchange Service, the Hertie Foundation, the Interdisciplinary Center for Clinical Studies (IZKF) Muenster, the German Foundation for Neurology, Almirall, Amicus Therapeutics, Germany, Biogen, Diamed, Fresenius Medical Care, Genzyme, Merck Serono, Novartis, ONO Pharma, Roche and Teva.A. Schnitzler has received lecture fees from Abbott, Novartis, Kyowa Kirin, and has been serving as a consultant for Abbott, Zambon, Medtronic Inc. He received royalties from the Georg Thieme Verlag. He is a government employee and receives through his institution funding for his research from the German Research Council, Abbott, and the Brunhilde Moll Foundation.S.J. Groiss received honoraria and/or travel expenses from Abbott Medical, Abbvie, Boston Scientific, Medtronic, Rogue Research, UCB, consulting fees from Zambon and research support from Abbott and Hilde-Ulrichs Stiftung. P. Albrecht received compensation for serving on Scientific Advisory Boards for Allergan, Celgene, Janssen Cilag, Ipsen, Merck, Merz Pharmaceuticals, Novartis, Biogen; he received speaker honoraria and travel support from Novartis, Teva, Biogen, Celgene, Merz Pharmaceuticals, Ipsen, Allergan, Bayer Healthcare, Esai, UCB; Roche; he received research support from Novartis, Allergan, Biogen, Celgene, Teva, Merz Pharmaceuticals, Ipsen, and Roche.

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CRediT authorship contribution statement

Carolin Balloff: Conceptualization, Investigation, Data curation, Methodology, Project administration, Formal analysis, Visualization, Writing – original draft, had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, share status as co-first authors. **Iris-Katharina Penner:** Conceptualization, Investigation, Funding acquisition, Methodology, Project administration, Writing – original draft, had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, share status as co-first authors. **Meng Ma:** Investigation, Writing – review & editing. **Iason Georgiades:** Investigation, Writing – review & editing. **Lina Scala:** Investigation, Writing – review & editing. **Nina Troullinakis:** Investigation, Writing – review & editing. **Jonas Graf:** Investigation, Writing – review & editing. **David Kremer:** Investigation, Writing – review & editing. **Orhan Aktas:** Investigation, Writing – review & editing. **Hans-Peter Hartung:** Investigation, Writing – review & editing. **Sven Günther Meuth:** Investigation, Writing – review & editing. **Alfons Schnitzler:** Resources, Writing – review & editing. **Stefan Jun Groiss:** Conceptualization, Investigation, Funding acquisition, Methodology, Project administration, Writing – original draft, had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, share status as co-last authors. **Philipp Albrecht:** Conceptualization, Investigation, Funding acquisition, Methodology, Project administration, Writing – original draft, had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, share status as co-last authors.

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Appendix A. Supplementary data

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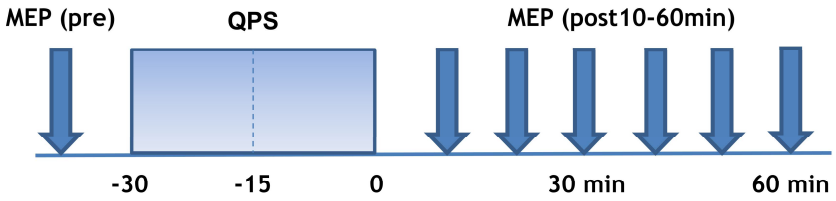
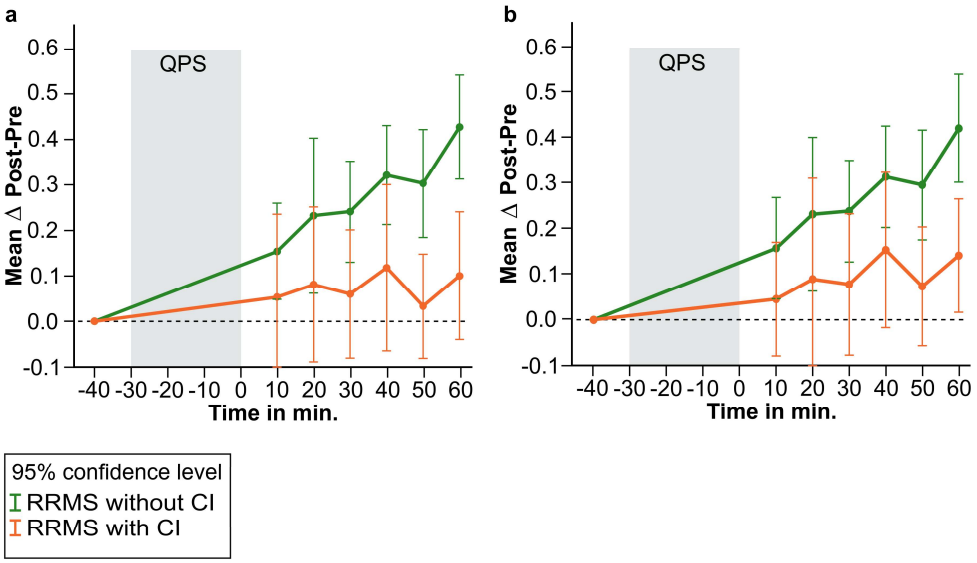
Fig. A1. Illustration of the QPS protocol.



Note. The figure represents the time course of the repetitive transcranial magnetic stimulation protocol. QPS = Quadripulse stimulation; MEP = Motor evoked potential.

Fig. A2. QPS-induced plasticity in RRMS patients with impairment in the SDMT (a) and BVMT-R (b) compared to patients without impairment in these tests.



Note. This figure shows the averaged difference between the pre and post QPS MEP amplitude per time point in RRMS patients with cognitive impairment. The left part (a) of the figure displays the data for patients with impairment in the SDMT and the right part (b) displays the data for patients with impairment in the BVMT-R. QPS = Quadripulse stimulation; RRMS = Relapsing-remitting Multiple Sclerosis; CI=Cognitive impairment; BVMT-R = Brief Visuospatial Memory Test Revised; SDMT= Symbol Digit Modalities Test.

Table A1. Characteristics of patients with and without cognitive impairment

Characteristic	$RRMS_{CI}$ (*N*=18)	$RRMS_{No\ CI}$ (*N*=45)	*p*-value
Sex, No. (%), female	8 (44)	34 (76)	**.04**
Handedness, No. (%), right	16 (89)	40 (89)	.42
Age, median (min-max), years	42 (23-61)	38 (20-55)	.15
Education, median (min-max), years	14 (11-19)	16 (8-22)	**.006**
AMT, median (min-max), % MSO	37.5 (33-52)	38 (27-73)	.80
RMT, median (min-max), % MSO	50 (37-67)	46 (33-81)	.22
MEP 0.5mV, median (min-max), % MSO	67.5 (44-100)	59 (36-100)	.13
MEP latency[a], median (min-max), ms	22.89 (17.74-37.83)	23.06 (18.60-31.42)	.46
ΔPost-Pre MEP amplitude, median (min-max), mV	0.32 (-0.10-1.32)	0.59 (-0.30-3.17)	.02
BVMT-R			
Total learning score, median (min-max)	12 (2-25)	28 (17-35)	**<.001**
z-score, median (min-max)[b]	-2.38 [-4.70-(-0.34)]	0.18 [-1.55-1.92]	**<.001**
SDMT			
correct items, median (min-max)	32.5 (19-67)	59 (38-84)	**<.001**
z-score, median (min-max)[c]	-2.19 (-4.12 - 0.70)	-0.08 (-1.62- 2.14)	**<.001**
HADS, No. (%), clinical[d]			
Anxiety	3 (17)	6 (13)	.71
Depression	3 (17)	6 (13)	.71
Disease duration, median (min-max), years	10.92 (3-30)	6.67 (0-27)	.12
EDSS, median (min-max)	3.0 (0-6.5)	1.5 (0-7.5)	.06
FSMC, No. (%), mild/moderate/severe[e]			
Motor	2 (11) / 2 (11) / 9 (50)	7 (16) / 6 (13) / 18 (40)	.90
Cognitive	2 (11) / 4 (22) / 5 (28)	6 (13) / 7 (16) / 16 (36)	.89
DMT exposure, No. (%)			.40
None	2 (11)	9 (20)	
Natalizumab	6 (33)	15 (33)	
Ocrelizumab	6 (32)	13 (29)	
Glatiramer acetate	2 (11)	0 (0)	
Dimethyl fumarate	1 (6)	1 (2)	
Interferon beta-1a	0 (0)	2 (4)	
Fingolimod	0 (0)	2 (4)	
Cladribine	0 (0)	2 (4)	
Alemtuzumab	1 (6)	0 (0)	
Teriflunomid	0 (0)	1 (2)	

Note. *p*-values < .05 are in boldface and based on two-tailed analysis. Number of impaired patients for each cognitive test and their combination: N_{SDMT}= 13; N_{BVMT}= 14; N_{Both}= 9. RRMS= Relapsing-remitting Multiple Sclerosis. CI= Cognitive impairment. AMT= Active Motor Threshold. RMT= Resting Motor Threshold. MEP 0.5mV= Stimulation intensity producing a reliable MEP of ~0.5mV. MSO= maximal stimulator output. BVMT-R= Brief Visuospatial Memory Test Revised. SDMT= Symbol Digit Modalities Test. HADS=Hospital Anxiety and Depression Scale. EDSS= Expanded Disability Status Scale. FSMC= Fatigue Scale of Motor and Cognition. Classification based on cut-off scores defined in the manual. DMT= Disease-modifying therapy.

[a] Missings as follow: 5 $RRMS_{CI}$, 1 $RRMS_{No\ CI}$.

[b] Calculation based on the BVMT-R manual[16].
[c] Calculation based on German norms.[17]
[d] Classification as clinical based on scores ≥ 11.
[e] Classification as mild, moderate, and severe based on cut-offs provided in the FSMC.[19]

Table A2. Multivariable linear mixed-effect model of MEP amplitude in cognitively impaired and unimpaired patients before and after QPS

Fixed Effects					**Random Effects**
	β-coefficient (95% CI)	*SE_b*	*t-value*	*p*	*s^2*
Intercept	0.57 (0.53; 0.62)[a]	0.02	25.20	**<.001**	
Pre QPS	Reference				
Post QPS	0.69 (0.52; 0.85)[a]	0.08	8.31	**<.001**	
No CI	Reference				
CI	-0.07 (-0.14; 0.01)	0.04	-1.80	.08	
Fatigue	-0.04 (-0.07; -0.00)[a]	0.02	-2.20	**.03**	
Post QPS*CI	-0.31 (-0.61; -0.02)[a]	0.15	-2.09	**.04**	
Subject*Pre QPS					0.00
Subject*Post QPS					0.33
Residual					0.01

Note. Two-tailed 95% CI and *p*-values are displayed. *p*-values <.05 are in boldface. QPS= Quadripulse Stimulation. CI= Cognitive Impairment. R^2(conditional)=0.95. R^2(marginal)=0.38. Adjusted Intraclass Correlation Coefficient=0.93.
[a] indicates statistical significance. *t*- and *p*-values are based on asymptotic Wald test.

Methods A1. Detailed description of linear mixed model computation

MEP amplitude was predicted based on the QPS intervention, group, QPS^{x}group interaction, and a random slope for the QPS effect, accounting for interindividual QPS response variability. A lag 1 autoregressive error structure controlled for significant autocorrelation in the data and continuous variables were centred at the sample mean. As we controlled the MEP amplitude to be ~0.5mV for all subjects prior to QPS, the variance differed substantially between time points. Therefore, a constant variance function structure with a grouping factor for the time of assessment (pre/post) was added to the model.

Selected covariates (age, depression, anxiety, fatigue, latency, education) and their interactions with QPS were added to the model and models were compared based on likelihood-ratio-tests or the Akaike information criterion when the former did not apply due to missing data in covariates. Collinearity was assessed by the variance inflation factor with a threshold of ≥ five as suggestive of multicollinearity. Based on these model statistics we report the best model. Coefficients were considered significant, if the 95% confidence interval did not include zero.

The same procedures were applied for the post-hoc computation of the model comparing cognitively impaired and cognitively preserved patients. Here, heteroscedasticity was not only observed between time points but also on the group level with higher variance in patients without CI than patients with CI. Therefore, a second grouping factor (CI/no CI) was added to the constant variance function structure.

For both models, the factor of interest was the QPS^{x}group interaction, as a significant interaction would indicate a stronger increase in MEP amplitude in one of the compared groups.

Study 2 – Synaptic plasticity during acute relapses

Balloff, C., Novello, S., Stucke, A.-S., Janssen, L. K., Heinen, E., Hartmann, C. J., Meuth, S. G., Schnitzler, A., Penner, I.-K.,[5] Albrecht, P.,[5] & Groiss, S. J.[5] (2023). Long-term potentiation-like plasticity is retained during relapse in patients with Multiple Sclerosis. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology, 155*, 76–85. https://doi.org/10.1016/j.clinph.2023.07.013

Conceptualization & methodology: I defined the research question and analysis plan in consultation with S.J. Groiss, P. Albrecht, and I.-K. Penner based on the experimental design, which was created by S.J. Groiss, P. Albrecht, and I.-K. Penner.

Investigation & project administration: I supervised recruitment of participants and data collection. I recruited participants and independently performed neuropsychological tests and rTMS. I was also responsible for data curation. Project administration was performed by me, S.J. Groiss, P. Albrecht, and I.-K. Penner. S.J. Groiss, P. Albrecht and C.J. Hartmann conducted neurological assessments. S. Novello, A.-S. Stucke, L.K. Janssen and E. Heinen recruited participants and conducted neuropsychological tests and rTMS under supervision. P. Albrecht and S.J. Groiss contributed to the recruitment of participants.

Formal analysis: I conducted the statistical analyses and reviewed them for correctness.

Resources: S.J. Groiss, P. Albrecht, and I.-K. Penner provided funding for this study. Further resources were provided by S.G. Meuth and A. Schnitzler.

Manuscript: I wrote the initial manuscript, which included all steps from comprehensive literature research to final formulation. I created the figures and tables with contributions of E. Heinen. I coordinated the scientific review process at the journal. During this process, with the assistance of I.-K. Penner, S.J. Groiss, and P. Albrecht, I made revisions. I prepared the final version of the manuscript. All authors critically reviewed the manuscript.

[5] Shared last authorship.

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Long-term potentiation-like plasticity is retained during relapse in patients with Multiple Sclerosis

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HIGHLIGHTS

- QPS-induced plasticity is retained during acute relapses in Multiple Sclerosis.
- Plasticity is higher in patients with motor disability than in patients without.
- Metaplasticity may be important to prevent motor disability in Multiple Sclerosis.

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ABSTRACT

Objective: To investigate the degree of synaptic plasticity in Multiple Sclerosis (MS) patients during acute relapses compared to stable MS patients and healthy controls (HCs) and to analyze its functional relevance.
Methods: Facilitatory quadripulse stimulation (QPS) was applied to the primary motor cortex in 18 acute relapsing and 18 stable MS patients, as well as 18 HCs. The degree of synaptic plasticity was measured by the change in motor evoked potential amplitude following QPS. Symptom recovery was assessed three months after relapse.
Results: Synaptic plasticity was induced in all groups. The degree of induced plasticity did not differ between acute relapsing patients, HCs, and stable MS patients. Plasticity was significantly higher in relapsing patients with motor disability compared to relapsing patients without motor disability. In most patients (n = 9, 50%) symptoms had at least partially recovered three months after the relapse, impeding meaningful analysis of the functional relevance of baseline synaptic plasticity.
Conclusions: QPS induced synaptic plasticity is retained during acute MS relapses. Subgroup analyses suggest that stabilizing metaplastic mechanisms may be more important to prevent motor disability but its functional relevance needs to be verified in larger, longitudinal studies.
Significance: New insights into synaptic plasticity during MS relapses are provided.

Abbreviations: MS, Multiple Sclerosis; RRMS, Relapsing-remitting Multiple Sclerosis; SPMS, Secondary-progressive Multiple Sclerosis; LTP, Long-term potentiation; LTD, Long-term depression; rTMS, Repetitive transcranial magnetic stimulation; QPS, Quadripulse stimulation; HCs, Healthy controls; TMS, Transcranial magnetic stimulation; FDI, First dorsal interosseous; MEP, Motor evoked potential; NHPT, Nine-hole peg test; T25FW, Timed 25-foot walk test; EDSS, Expanded Disability Status Scale; FSS, Functional System Scores; ΔMEP, Difference of the MEPs evoked pre and post QPS.

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1. Introduction

Multiple Sclerosis (MS) is the most prevalent demyelinating disease of the central nervous system (Love, 2006), affecting approximately 2.8 million people worldwide (Walton et al., 2020). Most patients (~80%) initially follow a relapsing-remitting course (RRMS), which can develop into secondary-progressive MS (SPMS). Attacks of new or abruptly aggravated neurological symptoms, which can fully recover, are the central element of RRMS and can also occur in SPMS. A minority of patients, (~20%) presents with primary progressive MS, which is characterized by a gradual increase of symptoms without any relapses (Lublin et al., 2014). MS typical inflammatory, demyelinating lesions can occur anywhere in the central nervous system, leading to a wide range of symptoms in all functional systems (Kurtzke, 1983). Mechanisms of compensatory reserve have been discussed to mediate the relationship between lesion load and disability. To summarize, a (limited) compensatory ability of the nervous system to attenuate or even prevent functional deficits has been described (Brandstadter et al., 2019; Krieger et al., 2016; Schoonheim et al., 2010).

In line with this, clinical recovery from relapse is highly variable with patients experiencing no, partial or complete remission (Mowry et al., 2009). According to a recent study, low levels of pre-existing disability and younger age at the time of relapse are both positively associated with complete recovery (Lublin et al., 2022). However, remyelination and processes of functional and structural reorganization may also play an important role for recovery due to their compensational potential (Tomassini et al., 2012). Reorganisation on the synaptic level via strengthening or weakening of synapses due to long-term potentiation (LTP) or long-term depression (LTD) is a central aspect of synaptic plasticity (Citri and Malenka, 2008). Both LTP and LTD can be safely modulated by repetitive transcranial magnetic stimulation (rTMS) and we have recently demonstrated that the degree of motor cortex plasticity induced by an excitability-increasing quadripulse stimulation (QPS) protocol (Hamada et al., 2008) may inform about the global synaptic plasticity in patients with RRMS during remission. While synaptic plasticity was not reduced in RRMS patients compared to healthy controls (HCs), cognitively impaired patients showed lower levels of QPS-induced plasticity than patients without cognitive deficits (Balloff et al., 2022).

Adaptive mechanisms of synaptic plasticity may also play an important role during relapse and have not yet been investigated in this phase of MS using QPS. Results from a previous study using a paired associated stimulation protocol suggest an association between the degree of LTP-like plasticity and clinical recovery (Mori et al., 2014). However, this finding could not be replicated by another research group using an LTD-inducing protocol (Wirsching et al., 2018). The latter study reported reversed effects of the LTD-protocol towards LTP-like effects during relapse but not during recovery three months later, which was interpreted as metaplastic effects during relapse (Wirsching et al., 2018). Hyperexcitability during relapse has also been described in another transcranial magnetic stimulation (TMS) study, which was, however, not interventional (Caramia et al., 2004). In contrast to this, our recent finding of reduced plasticity in cognitively impaired stable RRMS patients (Balloff et al., 2022) rather suggests reduced plasticity during relapse, since cognitive performance has been frequently described to be lower during relapses than during remission (Benedict et al., 2014; Benedict et al., 2020; Morrow et al., 2011).

We, therefore, aimed to investigate synaptic plasticity during relapse using QPS. As higher responder rates have been described for QPS compared to other rTMS protocols, it may produce more reliable results than previously implemented protocols (Guerra et al., 2017; Hamada et al., 2007; Nakamura et al., 2016; Tiksnadi et al., 2020). Our objective was to compare the degree of QPS-induced plasticity during relapse between patients with null, partial, or complete recovery. Based on the theoretical assumption of favorable effects of synaptic plasticity and the results described using another LTP-inducing protocol (Mori et al., 2014), we expected to find higher levels of QPS-induced plasticity in patients with complete compared to partial or null recovery. Patients with partial recovery were expected to present with more plasticity than patients with null recovery. Moreover, we aimed to compare the degree of QPS-induced plasticity in relapsing patients, HCs and stable patients. Based on previous results described above, we expected to find altered plasticity in relapsing patients compared to stable patients and HCs. Due to the ambiguous findings outlined above, no direction of this effect was hypothesized.

2. Methods

2.1. Subjects

Patients were recruited at the in- and outpatient neurological clinic of the University Hospital Düsseldorf, Germany, between May 2018 and October 2022. They had either been previously diagnosed with clinically definite MS or were newly diagnosed according to the 2017 revised McDonald criteria (Thompson et al., 2018). Subjects with a history of neurological or psychiatric diseases other than MS and remitted depressive episodes were excluded. Further exclusion criteria were contraindications for TMS and drug or alcohol abuse, which were addressed in a TMS safety screening questionnaire (Rossi et al., 2011). All eligibility criteria were tested using a standardized questionnaire. Relapse activity was defined according to the 2017 revised McDonald criteria (Thompson et al., 2018).

Patients in the stable control group had to be relapse-free for $\geq$ 90 days and were matched according to the following criteria: age, sex assigned at birth, education, disease duration, and disease type. HCs were matched based on age, sex assigned at birth, and education.

Prior to neuropsychological assessment, sufficient recognition of the visual material was tested for all patients describing visual deficits. Review and approval of the study was performed by the ethical committee of the medical faculty of the Heinrich Heine University Düsseldorf (study-number 2018–16) and informed written consent was obtained from all participants prior to participation. The study was carried out in accordance with the declaration of Helsinki.

2.2. Experimental design

Data were assessed according to the experimental design described in detail in our previous study (Balloff et al., 2022), which is summarized in Fig. 1. Briefly, subjects were neurologically and neuropsychologically examined before synaptic plasticity was assessed using the LTP-inducing QPS protocol, which was first introduced by Hamada et al. (2008). This protocol has also been implemented in our previous study (Balloff et al., 2022) as well as in other studies with HCs (Tamura et al., 2019; Tanaka et al., 2019) and neurologically diseased patients (Moriyasu et al., 2022). A total of 360 TMS-bursts of four monophasic TMS-pulses with a 5 ms interstimulus-interval were repeatedly administered at a frequency of 0.2 Hz for 30 minutes. Monophasic rTMS was established by four Magstim 200^2 stimulators (The Magstim Company Ltd., Whitland, UK), which were connected through a combining module from the same company. Stimulation intensity was established at 90% of the active motor threshold. Motor

evoked potentials (MEP) were adjusted to be ~ 0.5 mV pre QPS and recorded at the right first dorsal interosseous (FDI) muscle following single pulse monophasic TMS of the left primary motor cortex. Specifically, the FDI hotpot, i.e., the optimal position for eliciting the largest MEP in the FDI, was stimulated throughout the experiment.

Following 30 minutes of QPS intervention, MEP responses evoked by the same pre-interventional stimulation intensity were recorded every 10 minutes for a total of 60 minutes post QPS. At each time of assessment, 12 MEPs were recorded and averaged. Due to artefacts and/or voluntary muscle activity underlying the MEP response, which significantly exceeded the individual background activity, some MEPs were not considered for the averaged MEP amplitude. MEPs were discarded based on visual inspection of surface electromyography recordings by three independent investigators, resulting in exclusion of 1 MEP per time point and subject, on average.

During the TMS experiment, subjects were seated in a comfortable reclining chair with arms placed on cushioned armrests. A hand-held figure-of-eight coil (70 mm outer diameter. The Magstim Company Ltd., Whitland, UK) was used both for QPS as well as single-pulse TMS. Ag-AgCl electrodes (size: 20x15 mm, manufactured by Ambu, Ballerup, Denmark) in a belly-tendon montage were used to record MEPs using surface electromyography. The electromyography signal was amplified using a Digitimer D360 (Digitimer Ltd, Hertfordshire, UK) with a frequency band filter ranging from 100 to 5000 Hz. The amplified signal was digitized at a sampling rate of 5 kHz and analysed using Signal software (version 6.02, Cambridge Electronic Design Ltd., Cambridge, UK). Muscle activity was monitored in real-time on an oscilloscope (model DS1074B, Batronix Rigol, Preetz, Germany).

Neuropsychological assessment consisted of the Rao-adapted version of the Symbol Digit Modalities Test (Rao, 1990; Smith, 1982) as a measure of information processing speed and the Brief Visuospatial Memory Test Revised (Benedict, 1997) as a measure of visuospatial short-term memory and learning. Potential confounders for cognitive performance and synaptic plasticity, such as depression, anxiety and trait fatigue were measured with the Hospital Anxiety and Depression Scale (Herrmann-Lingen et al., 2011) and Fatigue Scale for Motor and Cognitive Functions (Penner et al., 2009). Motor function was assessed using the nine-hole peg test (NHPT) as a functional outcome of manual dexterity/fine motor skills and the timed 25-foot walk (T25FW) as a functional outcome of walking ability (Fischer et al., 2001). The degree of disability due to MS was assessed by the Expanded Disability Status Scale (EDSS; Kurtzke, 1983).

2.3. Statistical analyses

The number of patients was based on previous work using other rTMS protocols (Mori et al., 2014; Wirsching et al., 2018). Due to small sample sizes per group and non-normally distributed data, continuous clinical and demographic characteristics of the groups were compared using Mann-Whitney-U-test. Categorical data were compared using Fisher's exact test (IBM SPSS Statistics, version 28). In line with our previous approach in remitted RRMS patients and due to high reproducibility and standardization (Hamada et al., 2008), synaptic plasticity was operationalized by MEP amplitude changes after QPS.

The nlme package in R Studio (version 2022.02.3 + 492) was used to carry out linear mixed-effects models comparing QPS-induced motor plasticity between patients in an acute relapse and matched stable control patients as well as HCs. We used the same approach as in our previous study (Balloff et al., 2022) to compare the degree of induced excitability change between groups, while accounting for clustering of TMS assessments within subjects. Briefly, two MEPs entered analyses for each subject: 1) pre QPS MEP, controlled to be ~ 0.5 mV, and 2) maximum of the six mean post MEPs. Subsequently, MEP amplitudes were predicted based on the following fixed effects and a random slope of QPS intervention: QPS intervention (pre/post), group (relapsing MS/stable MS/ HCs), and QPS intervention^{x}group. To find the best fitting model, age, depression, anxiety, fatigue, and latency as well as their interactions with QPS were included and model performances were compared. Please refer to our previous study for statistical details on model computation (Balloff et al., 2022).

As we could not analyse the prognostic value of QPS-induced plasticity during relapse for the clinical outcome as planned (see results below), we post-hoc divided the relapse group in subgroups based on their Functional System Scores (FSS) during relapse. For each functional system except visual, bowel & bladder, and ambulation, two groups were created: 1) patients with at least mild disability, indicated by a FSS $\geq$ 2, and 2) patients with no disability, indicated by a FSS < 2. Plasticity was compared between relapse-subgroups using the same linear mixed-effects approach as described above, if there were at least n = 5 in both subgroups.

2.4. Data availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

3. Results

3.1. Neurological and neuropsychological profile

Out of 817 approached people, 18 patients in acute relapses were included (Fig. 2). Six of these patients were newly diagnosed with MS and two patients had already transitioned to SPMS. Accordingly, 18 matched stable patients (16 RRMS, 2 SPMS) and 18 HCs were included.

Table 1 shows the descriptive characteristics of all groups. Due to our matching procedure, groups did not differ with regards to any demographic characteristic. Further, relapsing and stable patients did not differ in terms of disease duration. However, patients in relapse were more severely disabled, as indicated by significantly higher EDSS scores. Compared to HCs, it also took them longer to complete the NHPT and the T25FW and they performed worse on the Symbol Digit Modalities Test. Neuropsychiatric symptoms, such as depression, anxiety and fatigue, were equally distributed in relapsing and stable patients. Despite more signs of fatigue in the relapsing group compared to HCs, no other differences were found between groups. On average, relapsing patients were assessed 21 days after symptom onset.

At the clinical follow-up three months after discharge, symptoms had partially remitted in most relapsing patients (n = 9, 50%). Only n = 3 (17%) had fully recovered, while n = 3 (17%) presented with no recovery at all. Three patients (17%) did not complete follow-up assessment.

3.2. Qps-induced plasticity in relapsing MS compared to stable MS and HCs

Fig. 3a shows the averaged difference of the MEPs evoked pre and post QPS (ΔMEP) for relapsing patients, stable patients, and HCs at each time of assessment (pre to post 6) with 95% confidence intervals. LTP-like effects were induced in all groups, as indicated by positive ΔMEP following QPS. However, large confidence intervals reveal high interindividual variance of the induced effects (please refer to Supplementary Figure 4 for individual raw MEP amplitudes over time), and the maximum averaged ΔMEP

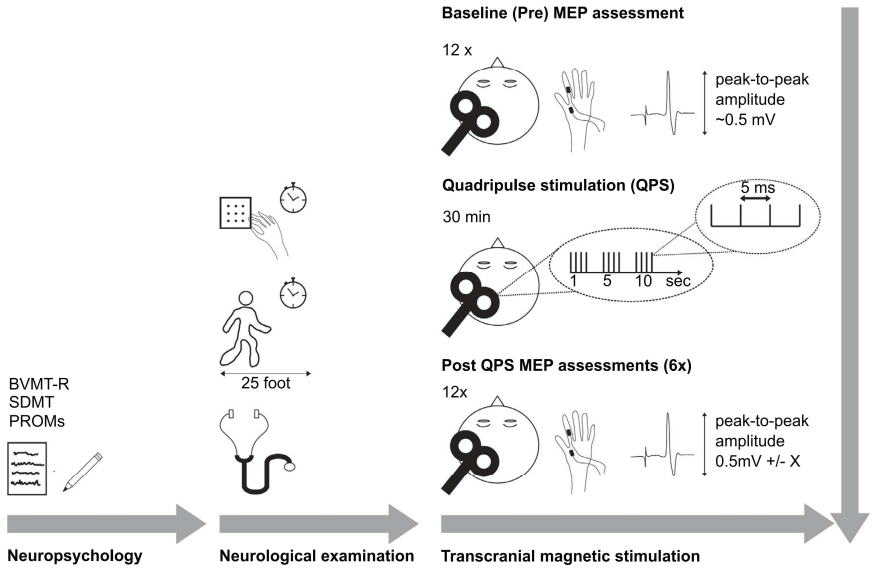

Fig. 1. Experimental design. This figure summarizes the experimental design. Assessment started with a short neuropsychological test battery and was followed by a neurological examination and transcranial magnetic stimulation. Details are described in our previous publication (Balloff et al., 2022). BVMT-R = Brief Visuospatial Memory Test Revised; SDMT = Symbol Digit Modalities Test; PROMs = Patient reported outcome measures; QPS = Quadripulse stimulation; MEP = Motor evoked potential.

occurred at different times of assessment across groups. While ΔMEP directly increased following QPS in HCs and stable MS, no increase was observed in relapsing patients until 20 min post QPS. In this group, ΔMEP reached its averaged maximum 60 min post QPS, whereas it peaked 40 min post QPS and remained constantly high in stable MS patients. In HCs, the averaged ΔMEP maximum also occurred 40 min post QPS. Despite these differences regarding the time course, the maximum averaged ΔMEP did not differ between groups.

This descriptive impression of equal degrees of plasticity in all groups was tested for statistical significance using linear mixed-effects models, accounting for interindividual variance in the after-effects of QPS. The best fitting model included QPS, group, latency, and QPS^{x}group with a conditional R^2 of 0.96 and a marginal R^2 of 0.42. In line with the experimental procedure to adjust MEP amplitudes to be ~ 0.5 mV prior to QPS, MEP pre amplitudes were equal across groups with only minimal deviations in stable patients (β = -0.04, p =.44) and HCs (β =+0.02, p =.72) compared to relapsing patients. Significant effects were revealed for QPS intervention (β = 0.48, p <.001) and latency (β = -0.07, p =.04; Table 2). As illustrated in Fig. 3b, the increase of MEP amplitude following QPS was comparable across groups. However, an adjusted intraclass correlation coefficient of 0.94 confirmed high inter-subject variability.

3.3. Clinical relevance of QPS-induced plasticity during relapse

With most patients (n = 9, 50%) experiencing partial recovery, no meaningful analysis of the functional relevance of induced plasticity was possible, since the other two groups (partial and full remission) consisted of only three patients each. To provide a descriptive impression of the data, baseline plasticity levels for each group are displayed in Supplementary Fig. 1. As described above, we divided the relapsing group into subgroups based on disability in the functional systems. The number of patients per group are presented in Supplementary Table 1.

Fig. 3c illustrates the averaged ΔMEP at each time of assessment (pre to post 6) with 95% confidence intervals for relapsing patients with and without motor disability (pyramidal FSS ≥ 2). While ΔMEP steadily increased in patients with motor disability, reaching its maximum 50 minutes post QPS, it remained more or less stable in relapsing patients without motor impairment. Linear mixed-effects modeling confirmed a significantly higher increase of MEP amplitude in patients with motor disability with an adjusted intraclass correlation coefficient of 0.90 and a satisfying fit (conditional R^2 = 0.95; marginal R^2 = 0.55). The full model is displayed in Supplementary Table 2 and demographic as well as clinical characteristics of the subgroups are presented in Supplementary Table 3.

No differences between subgroups were revealed for all other FSS (Supplementary Fig. 2).

4. Discussion

We present the first investigation of QPS-induced motor cortex plasticity during acute relapses in MS. Using this method, we did not observe altered levels of plasticity during relapse compared to stable MS and HCs. Importantly, however, plasticity was significantly higher in patients with motor disability compared to patients without motor disability.

This is of particular relevance as QPS-induced plasticity was measured at the motor cortex. In patients without motor disability, metaplastic effects may attenuate LTP in the motor cortex to avoid instability of the neuronal system (Galarreta and Hestrin, 1998) or even excitotoxicity (Abraham, 2008). Several mechanisms have been proposed to underlie metaplasticity, including alterations in: (1) synaptic strength and neurotransmitter release (Turrigiano

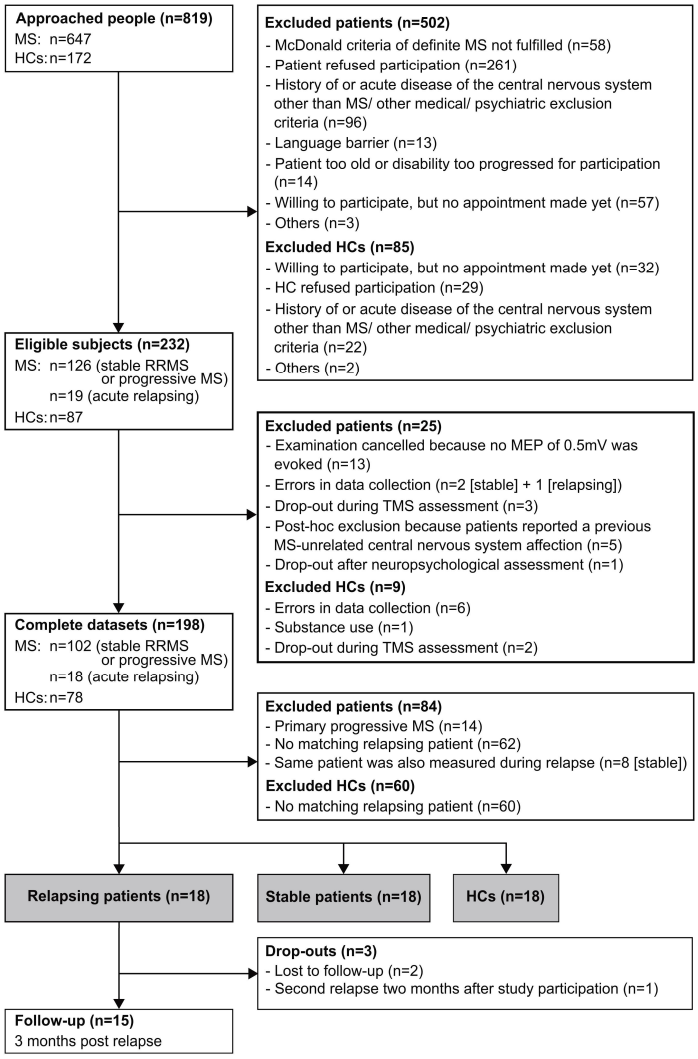

Fig. 2. Flowchart of the enrollment of subjects. This figure shows the numbers of subjects at each step of the study. HCs = Healthy controls; MS = Multiple Sclerosis; MEP = Motor evoked potentials; RRMS = Relapsing-remitting Multiple Sclerosis; TMS = Transcranial magnetic stimulation.

and Nelson, 2004), (2) neuromodulatory systems (Meunier et al., 2017), (3) signaling pathways (Kelleher et al., 2004), and (4) the activity of microglia and immune molecules (Wu et al., 2015). As we did not investigate any of these mechanisms in the present study, we can only speculate which of them may have altered synaptic plasticity in patients without motor disability. For example, increased activity of the motor cortex may have prevented both motor disability and induction of LTP by rTMS, as it has been shown that prior synaptic activity can inhibit LTP-induction (Coan et al., 1989). However, this is only one possible explanation, requiring confirmation in empirical studies.

Irrespective of the underlying mechanisms, the idea of metaplasticity as an important factor during MS relapses is supported by previous research. In fact, the previously reported reversed effects of an LTD-protocol in only mildly affected relapsing patients were also discussed to represent an adaptive response (Wirsching et al., 2018). Unfortunately, no details regarding the FSS of these patients were published, but based on the reported EDSS (median = 2.0) and main relapse symptoms (only n = 1 with motor symptoms), this cohort seems to resemble our subgroup of patients without motor disability. Taken together, this indicates that stabilizing metaplastic mechanisms may be key to prevent

Table 1
Sample characteristics.

Characteristic	Relapse (*N* = 18)	Stable (*N* = 18)	*p*-value	HCs (*N* = 18)	*p*-value
Sex, N (%), female	15 (83)	15 (83)	>0.99	15 (83)	>0.99
Handedness, N (%), right	16 (89)	17 (94)	>0.99	18 (100)	0.47
Age, Md (IQR), years	40 (15)	38.5 (13)	0.96	39 (17)	0.82
Education, Md (IQR), years	16 (4)	16 (4)	0.96	17.5 (5)	0.15
AMT, Md (IQR), % MSO	36 (12)	37 (12)	0.41	39 (7)	0.28
RMT, Md (IQR), % MSO	46 (18)	45 (16)	0.96	48 (9)	0.79
MEP 0.5 mV, Md (IQR), % MSO	57 (20)	58.5 (33)	0.65	58 (13)	0.83
MEP latency, Md (IQR), ms[a]	21.8 (4.0)	22.23 (3.4)	0.75	21.72 (2.5)	>0.99
ΔPost-Pre MEP amplitude, Md (IQR), mV	0.37 (0.6)	0.57 (0.6)	0.23	0.57 (0.3)	0.28
BVMT-R					
Total learning score, Md (IQR)	25.5 (11)	24.5 (8)	0.91	28.5 (6)	0.13
z-score, Md (IQR)[b]	−0.09 (1.9)	−0.28 (1.8)	0.91	0.50 (1.1)	0.14
SDMT					
correct items, Md (IQR)	50 (18)	59 (17)	0.21	59 (22)	**0.02**
z-score, Md (IQR)[c]	−0.75 (2.4)	0.24 (1.3)	0.20	−0.02 (2.1)	**0.01**
Nine-hole peg test					
Time to complete, Md (IQR), seconds[d]	21.0 (6.0)	19.1 (5.6)	0.12	18.6 (2.8)	**0.002**
25-foot walk test					
Time to complete, Md (IQR), seconds[e]	4.8 (6.2)	3.9 (1.2)	0.06	3.7 (1.0)	**0.01**
HADS, N (%), clinical[f]					
Anxiety	6 (33)	3 (17)	0.56	1 (6)	0.09
Depression	4 (22)	3 (17)	>0.99	0 (0)	0.10
FSMC, N (%), mild/moderate/severe[g]					
Motor	1 (6) / 1 (6) / 12 (67)	5 (28) / 0 (0) / 8 (44)	0.24	5 (28) / 1 (6) / 1 (6)	**<0.001**
Cognitive	2 (11) / 2 (11) / 10 (56)	5 (28) / 2 (11) / 6 (33)	0.48	3 (17) / 0 (0) / 1 (6)	**<0.001**
Disease duration, Md (IQR), years	6.5 (13)	6.2 (8)	0.86		
EDSS, Md (IQR)	4.0 (4.5)	1.0 (2.3)	**<0.001**		
DMT at time of assessment, N (%)			0.35		
None	9 (50)	3 (17)			
Natalizumab	1 (6)	6 (33)			
Ocrelizumab	4 (22)	5 (28)			
Glatiramer acetate	1 (6)	1 (6)			
Dimethyl fumarate	1 (6)	1 (6)			
Fingolimod	1 (6)	1 (6)			
Cladribine	1 (6)	1 (6)			
Days since relapse onset, Md (IQR)[h]	20.5 (17.8)				
Relapse treatment, N (%)					
None	3 (17)				
Glucocorticoids	5 (28)				
Plasmapheresis	2 (11)				
Glucocorticoids & Plasmapheresis	7 (39)				
Immunoadsorption	1 (6)				
Main relapse symptom, N (%)[i]					
Motor	10 (56)				
Sensible	11 (61)				
Visual	7 (39)				
Remission, N (%)[j]					
No	3 (17)				
Partial	9 (50)				
Full	3 (17)				
Days from relapse to follow-up, Md (IQR)[j]	109 (48)				

Note. *p*-values refer to the statistical comparison of the relapsing group against stable MS (first column) and against HCs (second column). *p*-values < 0.05 were considered significant and are in boldface. HCs = Healthy controls. MS = Multiple sclerosis. Md = Median. IQR = Interquartile range. AMT = Active motor threshold. RMT = Resting motor threshold. MEP = Motor evoked potential. MEP 0.5 mV = Stimulation intensity producing a reliable MEP of ∼ 0.5 mV. MSO = Maximal stimulator output. BVMT-R = Brief Visuospatial Memory Test Revised. SDMT = Symbol Digit Modalities Test. HADS = Hospital Anxiety and Depression Scale. FSMC = Fatigue Scale of Motor and Cognition. EDSS = Expanded Disability Status Scale. DMT = Disease-modifying therapy.

[a] Missing data: N = 3 (Relapse: N = 2, HCs: N = 1).
[b] Calculation based on the BVMT-R manual. (Benedict, 1997).
[c] Calculation based on German norms. (Scherer et al., 2004).
[d] Missing data: N = 3 (Relapse).
[e] For patients, who were unable to walk the required distance (N = 3, Relapse), the following time to complete was calculated: maximum time to complete in the total MS sample + 1.645*(standard deviation in the total MS sample). Missing data: N = 3 (Relapse).
[f] Classification as clinical based on scores ≥ 11.
[g] Classification as mild, moderate, and severe based on cut-offs provided in the FSMC. (Penner et al., 2009).
[h] Missing data: N = 2.
[i] Total does not add up to 100% because some patients presented with symptoms in more than one system.
[j] Missing data: N – 3. Percentages are based on the total sample including patients with missing data.

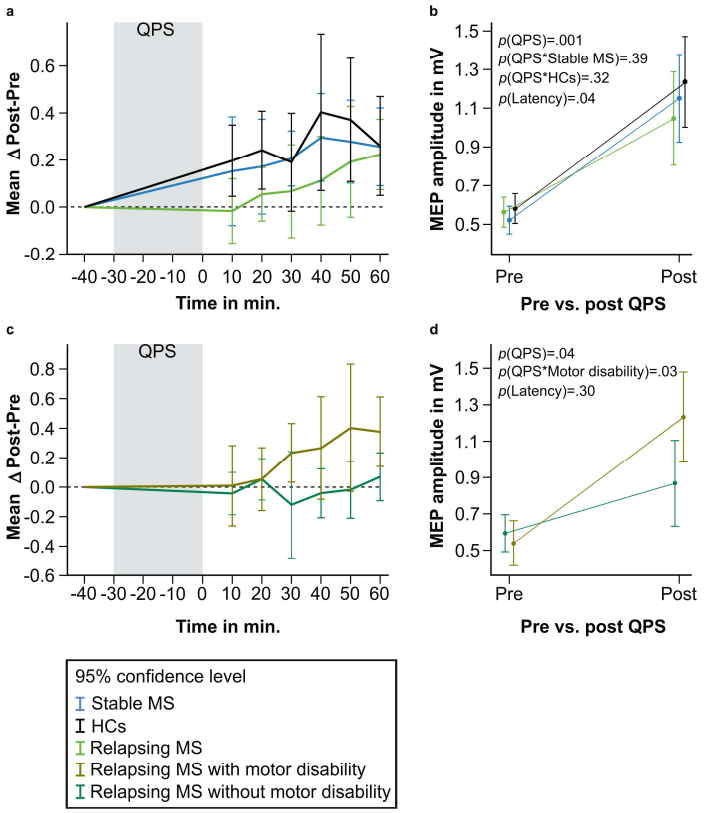

Fig. 3. QPS-induced plasticity in relapsing patients compared to matched stable patients and HCs (a, b) and in relapsing patients with motor disability compared to relapsing patients without motor disability (c, d). This figure displays the degree of QPS-induced plasticity in different clinical subgroups. The difference between the averaged MEP amplitude pre and post QPS over time is illustrated on the left side of the figure (a & c). On the right side of the figure (b & d), the predicted MEP amplitude are illustrated based on the fixed effects of the linear mixed models. The upper part of the figure (a & b) shows the comparison between relapsing patients, stable patients and HCs. No significant differences between groups were found. The lower part of the figure (c & d) displays QPS-induced plasticity in relapsing patients with and without motor disability. Relapsing patients without motor disability presented lower degrees of QPS-induced plasticity than relapsing patients with motor disability. QPS = Quadripulse stimulation; MEP = Motor evoked potential; HCs = Healthy Controls; MS = Multiple Sclerosis; ΔPost-Pre = Difference of the MEP evoked pre and post QPS.

Table 2
Multivariable linear mixed-effects model of MEP amplitude in HCs, relapsing, and stable MS patients before and after QPS.

Fixed Effects					**Random Effects**
	β-coefficient (95% CI)	*SE_b*	*t-value*	*p*	*s^2*
Intercept	+0.56 (0.48; 0.64)[a]	0.04	+14.25	**<0.0001**	
Pre QPS	Reference				
Post QPS	+0.48 (+0.24; +0.73)[a]	0.12	+3.97	**<0.001**	
Relapsing Patients	Reference				
Stable Patients	−0.04 (-0.15; +0.06)	0.05	−0.78	0.44	
HCs	+0.02 (-0.09; +0.13)	0.05	+0.36	0.72	
Latency	−0.07 (-0.14; −0.004)[a]	0.03	−2.12	**0.04**	
Post QPS*Stable Patients	+0.15 (-0.19; +0.48)	0.17	+0.87	0.39	
Post QPS*HCs	+0.17 (-0.17; +0.51)	0.17	+1.00	0.32	
Subject*Pre QPS					0.01
Subject*Post QPS					0.20
Residual					0.01

Note. Two-tailed 95% CI and *p*-values are displayed. *p*-values < 0.05 were considered significant and are in boldface. MEP = Motor evoked potential. HCs = Healthy controls. MS = Multiple sclerosis. QPS = Quadripulse stimulation. CI = Confidence interval. R^2(conditional) = 0.96. R^2(marginal) = 0.42. Adjusted Intraclass Correlation Coefficient = 0.93.
[a] indicates statistical significance. *t*- and *p*-values are based on asymptotic Wald test.

clinical (motor) disability during relapse, whereas synaptic plasticity itself may be secondary.

Dysfunctional hyperexcitability in the subgroup experiencing motor disability may also explain the significant group difference within the relapsing cohort. However, this is unlikely, as the degree of plasticity was similar in patients with motor disability and HCs. This indicates that the difference within the relapsing group rather reflects hypoexcitability in patients without motor disability than hyperexcitability in patients with motor disability.

Importantly, the reported FSS do not exclusively reflect disability during relapse but are also influenced by symptoms which were already present prior to the relapse. However, patients with a pyramidal FSS $\geq$ 2 presented motor symptoms during relapse significantly more often than patients with a pyramidal FSS < 2 (Supplementary Table 3). Thus, it is reasonable to assume that the FSS do not simply reflect pre-existing motor disability.

The fact that we did not find altered plasticity during relapse compared to stable MS and HCs may be explained by the clinical characteristics of the relapsing cohort. Despite moderate to severe disability and in contrast to other studies (Benedict et al., 2014; Benedict et al., 2020; Morrow et al., 2011), we did not find worse cognitive performance in relapsing compared to stable patients. As we did not perform repeated measurements, we cannot rule out cognitive decline in relapsing patients compared to performance prior to relapse. However, on average, all groups performed within the normative range. This suggests, that even in case of a slight cognitive deterioration, the decline did not reach clinical significance. Since only n = 6 (33%) of the relapsing patients experienced cognitive impairment, retained plasticity during relapse is in line with our previous finding of retained plasticity in stable RRMS patients without cognitive impairment (Balloff et al., 2022).

However, an average delay of 21 days after symptom onset, which is longer than in previous studies (Mori et al., 2014; Wirsching et al., 2018), may also explain why we did not find altered plasticity during relapse.

Earlier research on cortical excitability in patients with MS suggested increased MEP thresholds (Caramia et al., 2004), central motor conduction (Barker et al., 1986; Cowan et al., 1984; Hess et al., 1987), and cortical latency (Barker et al., 1986) compared to HCs. In the present study, MEP latency and thresholds were comparable between both patient groups and HCs. This might be explained by the patients' clinical characteristics. All patients included in two of the earlier studies had signs of upper limb involvement (Barker et al., 1986; Cowan et al., 1984). In our study, upper limb involvement was present in less than half of the patients, with only 15 patients affected across both groups. Taking into account the right side only, which was relevant for MEP assessment in our study, only 6 relapsing and 4 stable patients were affected. Hess et al. (1987) reported that prolonged central motor conduction was most strongly associated with hyperreflexia and brisk finger jerks and that the absence of these symptoms predicted normal central motor conduction. In our study, only n = 3 relapsing patients and n = 1 stable patient showed hyperreflexia, while none of them presented brisk finger jerks. Even though we did not assess central motor conduction, but only MEP latency, the absence of these symptoms in almost all our patients might explain why neither relapsing nor stable patients showed increased MEP thresholds or latency compared to HCs. Additionally, normal MEP thresholds have been described in two other studies on relapsing (Wirsching et al., 2018) and stable (Zeller et al., 2012) MS patients. In conclusion, MEP thresholds and latency indicate pyramidal tract affection, which is usually also accompanied by clinical signs. At the same time, MEPs do not seem to be altered in patients with MS without clinical pyramidal signs.

Even though treatment of relapse varied across subjects, most patients (n = 12, 67%) had received intravenous glucocorticoids prior to the examination or were still on treatment. This limits the generalizability of our findings, as glucocorticoids have been described to suppress LTP (Brandner et al., 2022; Dinse et al., 2017; Park et al., 2015). However, early glucocorticoid treatment represents the standard clinical care and could, therefore, not be postponed for study participation. The fact that the degree of induced plasticity during relapse did not differ compared to stable MS and HCs despite this possibly attenuating effect of glucocorticoids supports our finding of retained plasticity during relapse. Exploratively, we did not find significant differences regarding the degree of plasticity between patients who had received glucocorticoid treatment and those who had not, further supporting the validity of our results despite small subgroup sample size (n = 6 not receiving steroids; Supplementary Fig. 3).

Different rTMS protocols are hardly comparable, especially if they measure different types of synaptic plasticity (LTP vs. LTD). An acute relapse and MS in general may, in fact, not affect LTP and LTD in the same way. To analyze differential effects on different types of synaptic plasticity, future studies should investigate both LTP- and LTD-like effects in the same cohort, since focusing exclusively on only one of these types may disregard the complexity of synaptic plasticity.

A limitation of this study is the cross-sectional design. The relevance for clinical recovery could not be analyzed longitudinally because most patients presented with partial recovery, resulting in underrepresentation of the two other groups. Further, there was high variation within the relapse sample regarding the delay between symptom onset and study participation as well as disease duration. While some patients had been newly diagnosed with RRMS, others have been living with MS for up to 23 years. This variance may limit generalizability and future research should investigate homogeneous subgroups at different stages of the disease. Additionally, future research should aim at larger sample size per group to increase the reliability of the results.

We have averaged 12 MEPs at each timepoint for the primary outcome of MEP amplitudes to keep the protocol as short as possible, and therefore least exhausting for the participants. However, previous studies have averaged 20–30 MEPs to increase the reliability of MEP amplitudes (Chang et al., 2016; Goldsworthy et al., 2016) and, in general, standard errors reduce with increasing sample size. Therefore, averaging more MEPs per timepoint would have increased the accuracy of MEP amplitudes and, thus, the reliability of our results. Nonetheless, we believe that MEP amplitudes were estimated sufficiently accurate and that potential variations did not substantially influence our main findings, which should, however, be confirmed in future studies.

Despite these limitations, the strengths of our study are the closely matched and well characterized cohorts and the use of the QPS protocol. Results from other studies (Mori et al., 2014; Wirsching et al., 2018) did not reveal clear results, which may, at least partially, be explained by differences in TMS protocols. In fact, the protocol in the study reporting LTP-like effects of an LTD-inducing protocol during relapse did not induce any effects during recovery (Wirsching et al., 2018), questioning the interpretability of the results. Further, no information regarding the individual degree of recovery (null, partial, or full) was provided. It remains questionable, whether symptoms had recovered at follow-up, since optic neuritis was the main relapse symptom in n = 11 (58%) and is probably unrelated to the implemented indicator of recovery, namely performance in the Multiple Sclerosis Functional Composite Score (Wirsching et al., 2018). Lastly, both studies which have previously investigated plasticity during relapse, focused on newly diagnosed patients with low disability, with only one patient presenting an EDSS $\geq$ 4. This, however, resembles the median in our relapsing cohort, which also consisted of a substantial number of patients with severe disability.

5. Conclusion

We conclude that synaptic plasticity is retained during an acute MS relapse, even though standard treatment with glucocorticoids limits preclusion of possible hyperexcitability. Explorative subgroup analyses suggest that stabilizing metaplastic mechanisms may be more important to prevent motor disability than cortical excitability. The functional relevance of this finding needs to be investigated in larger, longitudinal studies.

CRediT authorship contribution statement

Carolin Balloff: Conceptualization, Investigation, Data curation, Methodology, Project administration, Formal analysis, Visualization, Writing – original draft. **Sveva Novello:** Investigation, Writing – review & editing. **Arved-Sebastian Stucke:** Investigation, Writing – review & editing. **Lisa Kathleen Janssen:** Investigation, Writing – review & editing. **Elisa Heinen:** Visualization, Writing – review & editing. **Christian Johannes Hartmann:** Investigation, Writing – review & editing. **Sven Günther Meuth:** Resources, Writing – review & editing. **Alfons Schnitzler:** Resources, Writing – review & editing. **Iris-Katharina Penner:** Conceptualization, Funding acquisition, Methodology, Writing – original draft. **Philipp Albrecht:** Conceptualization, Funding acquisition, Methodology, Writing – original draft. **Stefan Jun Groiss:** Conceptualization, Funding acquisition, Methodology, Writing – original draft.

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Conflict of Interest Statement

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S. Novello reports no disclosures relevant to the manuscript.

A-S. Stucke reports no disclosures relevant to the manuscript.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT (Version 3) in order to improve readability and linguistic style. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2023.07.013.

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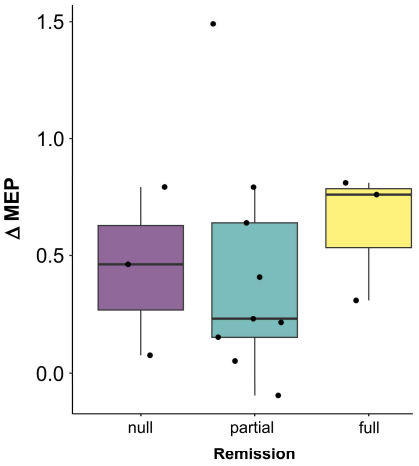
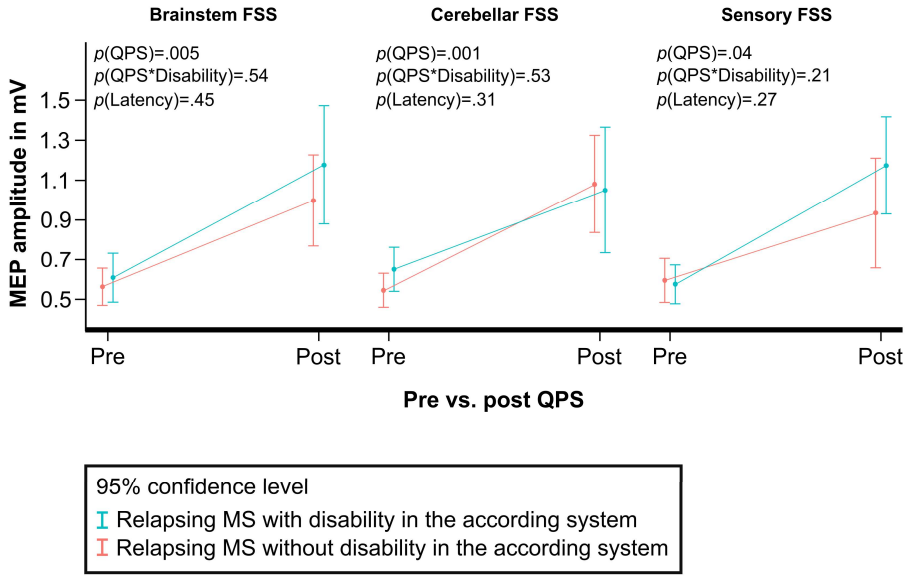


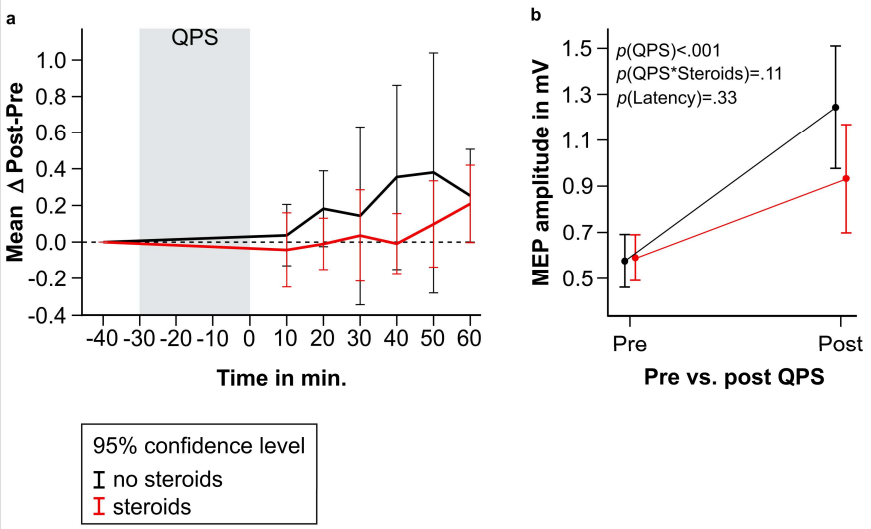
Supplementary Figure 1. QPS-induced plasticity in relapsing patients with different clinical recovery statuses

This figure illustrates the degree of QPS-induced plasticity in relapsing patients with null (left; n=3), partial (middle; n=9), or complete (right; n=3) remission on the group and individual level. Missing data regarding recovery status: n=3.
QPS= Quadripulse simuation; MEP= Motor evoked potential, ΔMEP= Difference of the MEP evoked pre QPS and the maximum averaged MEP post QPS.



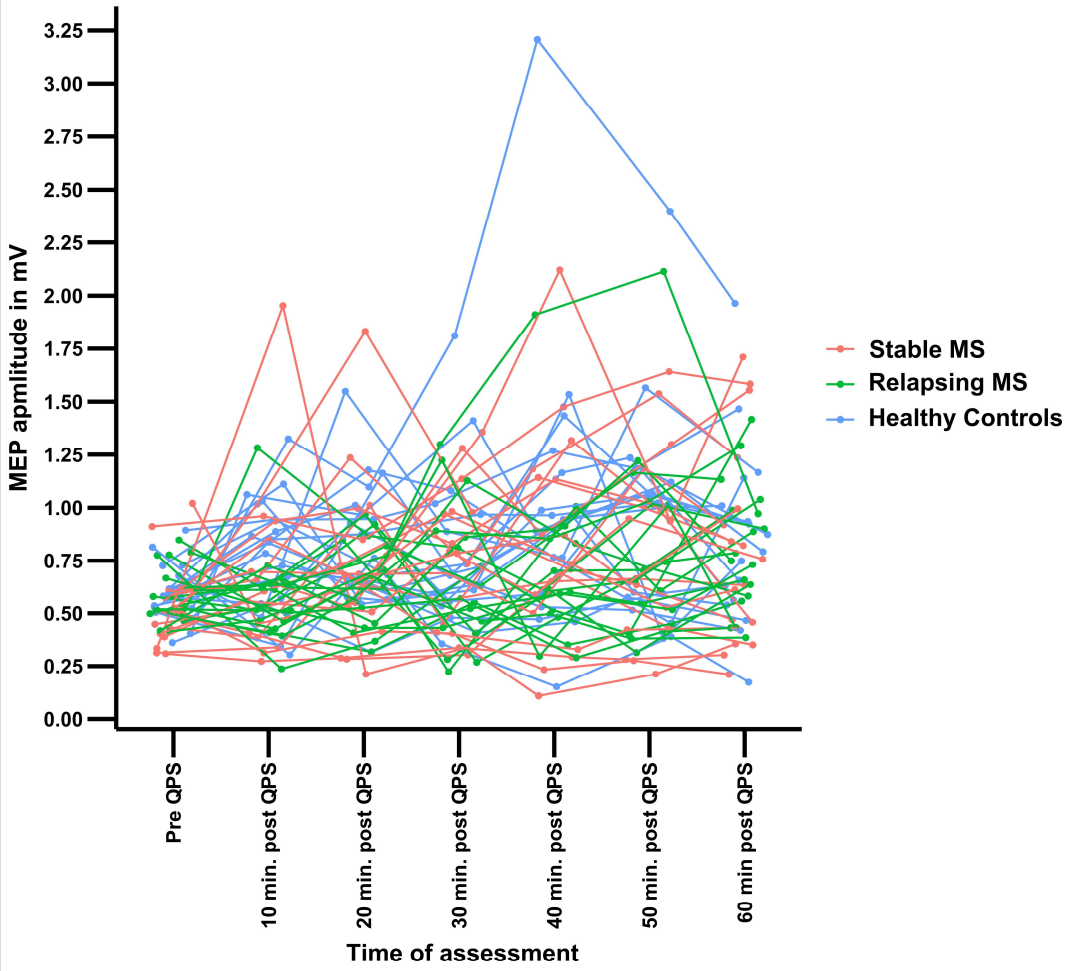
Supplementary Figure 2. QPS-induced plasticity in relapsing patients with different levels of disability during relapse.

This figure illustrates the degree of QPS-induced plasticity in relapsing patients based on the following FSS: brainstem, cerebellar, and sensory. For each FSS, patients with and without disability in the according system were compared using linear mixed-effects models. The predicted MEP amplitudes pre and post QPS based on the fixed effects of these models are displayed. Disability was defined as a FSS ≥2 in the according system. No comparison between patients with and without disability in the cerebral functional system was conducted, because only n=2 presented with disability (see Supplementary Table 1).
QPS= Quadripulse stimulation; MEP= Motor evoked potential; FSS= Functional System Score; MS= Multiple Sclerosis.



Supplementary Figure 3. QPS-induced plasticity in relapsing patients receiving glucocorticoid treatment compared to relapsing patients not receiving glucocorticoid treatment

This figure illustrates the degree of QPS-induced plasticity in relapsing patients who received glucocorticoid treatment prior or during assessment (n=12) compared to those who did not (n=6). Part a displays the difference between the averaged MEP amplitude pre and post QPS over time, while part b shows the predicted MEP amplitude based on the fixed effects of a linear mixed-effects model.
QPS= Quadripulse stimulation; MEP= Motor evoked potential; DPost-Pre= Difference of the MEP evoked pre and post QPS



Supplementary Figure 4. Raw data of MEP amplitude over time

This figure illustrates the MEP amplitudes in mV per subject and group at each time of assessment. Data points of the same subject are connected by lines and subjects of the same group are displayed in the same colour (blue= healthy controls, red= stable MS, green= relapsing MS). QPS= Quadripulse stimulation; MEP= Motor evoked potential; MS= Multiple Sclerosis.

Supplementary Tables

Supplementary Table 1. Distribution of disability in the Functional Systems

Functional System	Disability (FSS ≥ 2)	No disability (FSS < 2)
Brainstem (N, %)	7 (39)	11 (61)
Pyramidal (N, %)	9 (50)	9 (50)
Cerebral (N, %)	2 (11)	16 (89)
Sensory (N, %)	9 (50)	9 (50)
Cerebellar (N, %)	6 (33)	12 (67)

Note. Visual, bowel and bladder, and ambulation are not displayed because no association with cortical plasticity is expected. FSS= Functional System Score.

Supplementary Table 2. Multivariable linear mixed-effects model of MEP amplitude in relapsing patients with and without motor disability before and after QPS

Fixed Effects					Random Effects
	β-coefficient (95% CI)	*SE_b*	*t-value*	*p*	*s^2*
Intercept	+0.59 (+0.48; +0.70)[a]	0.05	+11.44	**<.001**	
Pre QPS	Reference				
Post QPS	+0.27 (+0.01; +0.54)[a]	0.12	+2.24	**.04**	
No motor disability	Reference				
Motor disability	-0.06 (-0.23; +0.12)	0.08	-0.67	.51	
Latency	-0.06 (-0.18; +0.06)	0.06	-1.08	.30	
Post QPS*Motor disability	+0.42 (+0.05; +0.79)	0.17	+2.42	**.03**	
Subject*Pre QPS					0.01
Subject*Post QPS					0.09
Residual					0.01

Note. Two-tailed 95% CI and *p*-values are displayed. *p*-values <.05 were considered significant and are in boldface. Motor disability was defined as a pyramidal Functional System Score ≥ 2. QPS= Quadripulse stimulation. R^2(conditional)=0.95. R^2(marginal)=0.55. Adjusted Intraclass Correlation Coefficient=0.90.

[a] indicates statistical significance. *t*- and *p*-values are based on asymptotic Wald test.

Supplementary Table 3. Characteristics of relapsing patients with and without motor disability

Characteristic	Motor disability (*N*=9)	No motor disability (*N*=9)	*p*-value
Sex, N (%), female	9 (100)	6 (67)	.21
Handedness, N (%), right	9 (100)	7 (78)	.47
Age, Md (IQR), years	42 (16)	35 (15)	.11
Education, Md (IQR), years	16 (3)	16 (5)	.26
AMT, Md (IQR), % MSO	36 (19)	35 (8)	.93
RMT, Md (IQR), % MSO	47 (25)	44 (12)	.61
MEP 0.5mV, Md (IQR), % MSO	58 (29)	52 (14)	.55
MEP latency, Md (IQR), ms[a]	20.7 (3.3)	23.0 (5.0)	.13
ΔPost-Pre MEP amplitude, Md (IQR), mV	0.7 (0.4)	0.2 (0.3)	**.03**
BVMT-R			
Total learning score, Md (IQR)	22 (13)	26 (11)	.19
z-score, Md (IQR)[b]	-0.4 (2.6)	0.4 (1.8)	.26
SDMT			
correct items, Md (IQR)	45 (25)	53 (20)	.16
z-score, Md (IQR)[c]	-0.7 (3.0)	-0.8 (2.2)	.67

Nine-hole peg test			
Time to complete, Md (IQR), seconds[d]	25.8 (8.6)	20.5 (3.9)	.15
25-foot walk test			
Time to complete, Md (IQR), seconds[e]	10.0 (5.9)	4.0 (1.2)	**.001**
HADS, N (%), clinical[f]			
Anxiety	3 (33)	3 (33)	>.99
Depression	2 (22)	2 (22)	>.99
FSMC, N (%), mild/moderate/severe[g]			
Motor	0 (0) / 1 (11) / 8 (89)	1 (11) / 0 (0) / 4 (44)	.05
Cognitive	0 (0) / 1 (11) / 8 (89)	2 (22) / 1 (11) / 2 (22)	**.02**
Disease duration, Md (IQR), years	11.9 (16)	0.1 (6)	**.003**
EDSS, Md (IQR)	6.5 (2.0)	2.0 (2.5)	**<.001**
DMT at time of assessment, N (%)			**.002**
None	1 (11)	8 (80)	
Natalizumab	1 (11)	0 (0)	
Ocrelizumab	4 (44)	0 (0)	
Glatiramer acetate	0 (0)	1 (10)	
Dimethyl fumarate	1 (11)	0 (0)	
Fingolimod	1 (11)	0 (0)	
Cladribine	1 (11)	0 (0)	
Days since relapse onset, Md (IQR)[h]	20.5 (10)	18.5 (20)	.51
Relapse treatment, N (%)			.45
None	1 (11)	2 (22)	
Glucocorticoids	2 (22)	3 (33)	
Plasmapheresis	2 (22)	0 (0)	
Glucocorticoids & Plasmapheresis	3 (33)	4 (44)	
Immunoadsorption	1 (11)	0 (0)	
Main relapse symptom, N (%)[i]			
Motor	8 (89)	2 (22)	**.02**
Sensible	5 (56)	6 (67)	>.99
Visual	2 (22)	5 (56)	.33
Remission, N (%)[j]			.70
No	2 (22)	1 (11)	
Partial	4 (44)	5 (55)	
Full	1 (11)	2 (22)	
Days from relapse to follow-up, Md (IQR)[j]	96 (48)	122.5 (81.8)	.34

Note. *p*-values <.05 were considered significant, are in boldface, and refer to the two-tailed comparison against patients in relapse. Motor disability was defined as a pyramidal Functional System Score ≥ 2. HCs= Healthy controls. Md=Median. IQR=Interquartile range. AMT= Active Motor Threshold. RMT= Resting Motor Threshold. MEP= Motor evoked potential. MEP 0.5mV= Stimulation intensity producing a reliable MEP of ~0.5mV. MSO= Maximal stimulator output. BVMT-R= Brief Visuospatial Memory Test Revised. SDMT= Symbol Digit Modalities Test. HADS=Hospital Anxiety and Depression Scale. FSMC= Fatigue Scale of Motor and Cognition. EDSS= Expanded Disability Status Scale. DMT= Disease-modifying therapy. MS= Multiple Sclerosis.

[a] Missing data: N=2 (motor impairment: N=1, no motor impairment: N=1)

[b] Calculation based on the BVMT-R manual (Benedict, 1997).

[c] Calculation based on German norms (Scherer et al., 2004).

[d] Missing data: N=3 (motor disability: N=2, no motor disability: N=1)

[e] For patients, who were unable to walk the required distance (N=3, motor disability), the following time to complete was calculated: maximum time to complete in the total MS Sample + 1.645*(standard deviation in the total MS sample). Missing data: N= 3 (motor disability: N=2, no motor disability: N=1).

[f] Classification as clinical based on scores ≥ 11.

[g] Classification as mild, moderate, and severe based on cut-offs provided in the FSMC (Penner et al., 2009).

[h] Missing data: N=2 (motor disability: N=1, no motor disability: N=1)
[i] Total does not add up to 100% because some patients presented with symptoms in more than one system. % based on the total number of subjects per group.
[j] Missing data: N=3 (motor disability: N=2, no motor disability: N=1)

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Study 3 – Synaptic plasticity in PMS and its association with motor performance

Balloff, C.,[6] Albrecht, P.,[6] Stucke, A.-S., Scala, L., Novello, S., Hartmann, C. J., Meuth, S. G., Schnitzler, A., Penner, I.-K.,[7] & Groiss, S.J.[7] (2023). The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis. *Frontiers in Neurology, 14,* Article 1266225. https://doi.org/10.3389/fneur.2023.1266225

Conceptualization & methodology: I defined the research question and analysis plan in consultation with S.J. Groiss, P. Albrecht, and I.-K. Penner based on the experimental design, which was created by S.J. Groiss, P. Albrecht, and I.-K. Penner.

Investigation & project administration: I supervised recruitment of participants and data collection. I recruited participants and independently performed neuropsychological tests and rTMS. I was also responsible for data curation. Project administration was performed by me, S.J. Groiss, P. Albrecht, and I.-K. Penner. S.J. Groiss, P. Albrecht and C.J. Hartmann conducted neurological assessments. A.-S. Stucke, L. Scala, S. Novello recruited participants and conducted neuropsychological tests and rTMS under supervision. A.-S. Stucke provided support in data curation. P. Albrecht and S.J. Groiss contributed to the recruitment of participants.

Formal analysis: I conducted the statistical analyses with contributions by A.-S. Stucke and reviewed them for correctness.

Resources: I provided funding for the open access fee. P. Albrecht provided funding for this study. Further resources were provided by S.G. Meuth and A. Schnitzler.

Manuscript: I wrote the initial manuscript, which included all steps from comprehensive literature research to final formulation. I created the figures and tables. I coordinated the scientific review process at the journal. During this process, with the assistance of I.-K. Penner, S.J. Groiss, and P. Albrecht, I made revisions. I prepared the final version of the manuscript. All authors critically reviewed the manuscript. I.-K. Penner, S.J. Groiss, and P. Albrecht additionally contributed to the original draft.

[6] Shared first authorship.
[7] Shared last authorship.

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The importance of pyramidal tract integrity for cortical plasticity and related functionality in patients with multiple sclerosis

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Background: Cortical plasticity induced by quadripulse stimulation (QPS) has been shown to correlate with cognitive functions in patients with relapsing-remitting multiple sclerosis (RRMS) and to not be reduced compared to healthy controls (HCs).

Objective: This study aimed to compare the degree of QPS-induced plasticity between different subtypes of multiple sclerosis (MS) and HCs and to investigate the association of the degree of plasticity with motor and cognitive functions. We expected lower levels of plasticity in patients with progressive MS (PMS) but not RRMS compared to HCs. Furthermore, we expected to find positive correlations with cognitive and motor performance in patients with MS.

Methods: QPS-induced plasticity was compared between 34 patients with PMS, 30 patients with RRMS, and 30 HCs using linear mixed-effects models. The degree of QPS-induced cortical plasticity was correlated with various motor and cognitive outcomes.

Results: There were no differences regarding the degree of QPS-induced cortical plasticity between HCs and patients with RRMS ($p = 0.86$) and PMS ($p = 0.18$). However, we only found correlations between the level of induced plasticity and both motor and cognitive functions in patients with intact corticospinal tract integrity. Exploratory analysis revealed significantly reduced QPS-induced plasticity in patients with damage compared to intact corticospinal tract integrity ($p < 0.001$).

Conclusion: Our study supports the notion of pyramidal tract integrity being of more relevance for QPS-induced cortical plasticity in MS and related functional significance than the type of disease.

KEYWORDS

cortical plasticity, motor function, repetitive transcranial magnetic stimulation, quadripulse stimulation, pyramidal tract integrity

1 Introduction

Neuroplasticity represents an important mechanism of the human brain to overcome disease-induced changes and impairment in the communication of neuronal networks. It does not only facilitate learning and memory (1) but also environmental adaptation and thus reflects an indispensable prerequisite for recovery and rebuilding of neuronal connections after brain injury and brain disease (2).

In multiple sclerosis (MS), a decline in motor and cognitive performance is the consequence of increased structural damage, finally leading to a network collapse impeding the brain's capability to reorganize (3). Thus, interventions able to promote brain plasticity to regain and/or preserve functions are of tremendous clinical and scientific interest and need. However, potential therapeutic interventions can only be investigated using reliable biomarkers of plasticity with high functional relevance, one of which may be repetitive transcranial magnetic stimulation (rTMS). Depending on the applied frequency, rTMS can change neural excitability by inducing effects similar to long-term potentiation (LTP) and long-term depression (LTD) (4). Many rTMS protocols exist and a protocol called quadripulse stimulation (QPS) (5) is supposed to promote LTP in healthy subjects with the lowest variability (6–8).

Recently, we were able to show that cortical plasticity can be induced by QPS of the motor cortex in patients with relapsing-remitting MS (RRMS) (9). In this cohort, plasticity induced by our QPS protocol was significantly associated with information processing speed, visuospatial learning and short-term memory, and with clinical disability. Correspondingly, cortical plasticity was higher in subjects with preserved cognitive function than in those presenting cognitive deficits. Compared to healthy controls (HCs), our overall mildly affected group of RRMS patients presented with similar levels of cortical plasticity (9).

Even though these findings indicate that QPS-induced plasticity could reflect global synaptic plasticity beyond the motor cortex, research is actually limited to patients with RRMS, neuropsychological performance, and clinical disability. Thus, to extend our knowledge and understanding in terms of clinical relevance and prognostic value of the QPS method, it is required to study its potential in different disease types and its relevance for motor functions as well.

In the present study, we, therefore, analyzed the correlation between the degree of synaptic plasticity with motor functions of the upper and lower extremities as well as with cognitive outcomes for processing speed and visuospatial short-term memory and learning in different types of MS and matched HCs. Furthermore, we compared the degree of QPS-induced plasticity between HCs and different MS subtypes. Based on the previously described association of cortical plasticity and clinical disability in patients with RRMS (9), we hypothesized QPS-induced plasticity to positively correlate with motor outcomes across all disease types. Based on the results from our first RRMS cohort, we further expected to find positive correlations with cognitive performance in patients with progressive MS (PMS).

To the best of our knowledge, only one study has investigated LTP- or LTD-like plasticity induced by rTMS in patients with PMS so far (10). Plasticity was shown to be reduced in patients with primary progressive MS (PPMS) compared to stable patients with RRMS and HCs. Patients with PPMS neither showed LTP-like effects following intermittent theta burst stimulation (iTBS) nor LTD-like effects following continuous theta burst stimulation (cTBS) (10). It was argued that these findings may be due to excitotoxicity neuronal damage and loss of a sufficient LTP-like response in patients with PPMS (11).

Even though these findings indicate that reduced or even absent synaptic plasticity may be an important factor driving clinical deterioration in patients with PPMS, the sample size of the PPMS group was too small ($n = 12$) to generalize the findings, and both iTBS and cTBS typically show high rates of non- or even opposite responders as well as high intra- and inter-individual variability (12). Furthermore, no data on the degree of cortical plasticity in patients with secondary progressive MS (SPMS) have been published until now.

Despite limited comparability between different rTMS protocols, both the aforementioned protocols and QPS aim to induce either LTP or LTD. Thus, in line with the previous findings and the fact that patients with PMS typically express more disability than patients with RRMS (13, 14), we expected to find similar results using QPS as in the previous study (10). Specifically, we hypothesized plasticity to be reduced in patients with PMS but not in RRMS compared to HCs.

2 Materials and methods

The design and methods of the study have been described in detail elsewhere (9). In the following paragraphs, we therefore only summarize the most relevant information to understand the design of the study as well as any deviations from our previous publication.

2.1 Subjects

Data were collected between May 2018 and October 2022 at the Department of Neurology at the University Hospital in Düsseldorf, Germany. The inclusion criterion was a diagnosis of definite MS according to the revised McDonald criteria (15). The exclusion criteria were as follows: (1) history of diseases of the central or peripheral nervous system other than MS, (2) history of psychiatric diseases potentially affecting cognition other than remitted depressive episodes, (3) presence of any contraindication for transcranial magnetic stimulation (TMS), (4) history of drug or alcohol abuse, (5) age of <18 years. Based on the same exclusion criteria, age-, sex-, and education-matched HCs were recruited from an internal database of interested HCs as well as friends and family members of faculty members of the University Hospital Düsseldorf. A TMS safety screening questionnaire (16) was carried out, and informed written consent was obtained by all persons before participation. The ethical committee of the medical faculty of the Heinrich Heine University Düsseldorf (study number 2018-16) reviewed and approved the study, which was carried out in accordance with the Declaration of Helsinki.

2.2 Experimental design and data assessment

Details of the experimental design have been described in our previous publication (9). To summarize, a short neuropsychological assessment, including the Rao-adapted version of the Symbol Digit Modalities Test (SDMT) (17, 18), the Brief Visuospatial Memory Test-Revised (BVMT-R) (19), and patient-reported outcome measures of fatigue (Fatigue Scale for Motor and Cognitive Functions) (20), depression, and anxiety (Hospital Anxiety and Depression Scale) (21), was administered. Furthermore, the nine-hole peg test (NHPT) was applied as a functional outcome of manual dexterity, and the timed 25-foot walk test (T25FW) served as a measure of ambulation (22). The Expanded Disability Status Scale (EDSS) was determined by an experienced neurologist as an indicator of overall disability (23).

Change in motor-evoked potential (MEP) amplitudes at the right first dorsal interosseous muscle following 30 min of QPS-5 stimulation (5) served as a measure of LTP-like synaptic plasticity. MEPs were evoked by single-pulse monophasic TMS and were adjusted to be ∼0.5 mV before the QPS-5 intervention to ensure comparability across subjects. In total, 12 MEPs were averaged for analysis. The same stimulation intensity was used to record MEPs post-QPS intervention for a total of 60 min. An average of 12 MEPs was calculated. However, on average, one MEP per subject was excluded at each time of assessment due to voluntary muscle activity and/or artifacts.

To assess pyramidal tract integrity, MEP latency was measured by single-pulse TMS. Participants were told to maintain a contraction of ∼30% of the maximum voluntary activity at the target muscle for 10 consecutive trials, while they were stimulated with an intensity of 140% of their individual active motor threshold. The mean latency of the ten trials was used for analyses (24).

2.3 Statistical analyses

Since there are no published data on QPS-induced plasticity in patients with PMS so far, the number of enrolled subjects was based on the number of eligible patients with PMS and matched patients with RRMS/HCs. The comparison of demographic and clinical characteristics was conducted using IBM SPSS Statistics (version 28). All other analyses were carried out in R studio (version 2022.12.0), and statistical tests were considered significant based on $\alpha < 0.05$.

Clinical and demographic characteristics were compared between groups using the Kruskal–Wallis test for continuous variables because data were non-normally distributed in at least one group per variable. MS-specific continuous characteristics (e.g., EDSS) were compared between patients with RRMS and PMS using the Mann–Whitney *U*-test due to non-normal distribution. Fisher's exact test was used to compare categorical data between groups. Significant omnibus tests were followed by Dunn's test or pairwise Fisher's exact test to identify which specific group(s) differed from the others. We report significant pairwise group differences based on uncorrected and Bonferroni–Holm-corrected *p*'-values (25).

To improve standardization and ensure comparability across our studies, the maximum change in MEP amplitude after QPS was used as our measure of synaptic plasticity.

Due to the presence of outliers, associations between QPS-induced plasticity and functional readouts (BVMT-R, SDMT, NHPT, and T25FW) were investigated by Spearman's rank correlation coefficients of these measures with the difference between the maximum of the six mean post-MEPs and the pre-MEP amplitude (ΔMEP) separately in each group. Uncorrected one-tailed *p*-values and Bonferroni–Holm-corrected *p*'-values are reported (25, 26). Data inspection revealed no clear linear relationship between the NHPT, T25FW, and ΔMEP in either group. Therefore, we did not further analyze linear relationships for these parameters but used generalized additive models (GAMs) to explore more complex linear and non-linear relationships with the "mgcv" package in both patient groups. In addition, we explored the following types of splines: thin plate, penalized cubic, cyclic cubic, shrinkage cubic, and p. Models were compared against the regular linear model using the "anova" function.

Linear mixed-effects models were calculated using the "nlme" package to compare the degree of induced plasticity between HCs, patients with RRMS, and patients with PMS. In line with our previous study (9), the increase of MEP amplitude following QPS was analyzed by comparing the maximum of the six mean post-MEP amplitude against the mean MEP amplitude before QPS (∼0.5 mV). A random slope for the intervention (pre/post-QPS) was added to the fixed effects of the intervention (pre/post-QPS), group (HCs, RRMS, and PMS), and their interaction. This accounted for both the dependency of pre- and post-MEP amplitudes within subjects due to repeated measurements as well as for the variability of the interventional effect.

The basic model predicting the MEP amplitude included the fixed effects of the intervention (pre/post-QPS) and group (HCs, RRMS, and PMS), as well as their interaction. To account for the subject-dependent variability in response to the intervention, a random slope for the intervention (pre/post-QPS) was included. For our research question, the interactions of *post-QPS*group* were most relevant since significant interactions would indicate a significant difference in the degree of plasticity between the corresponding groups. Specifically, we hypothesized a significantly reduced increase of MEP amplitude in patients with PMS compared to HCs and patients with RRMS. Due to this directed hypothesis, one-tailed confidence intervals and *p*-values were conducted for the factor *post-QPS*PMS*. All other confidence intervals and *p*-values were based on two-tailed analysis.

In line with our previous study (9), age, depression, anxiety, fatigue, and MEP latency, as well as their interactions were separately added to the model. Models including covariates were tested against the basic model described above based on likelihood-ratio tests. Models including covariates with missing data were compared based on the Akaike information criterion. The variance inflation factor (cutoff value of ≥5) was used to investigate collinearity. All models were estimated using the "restricted maximum likelihood method" because it results in a more precise estimation of standard errors in smaller samples (27). We only report the model with the best fit.

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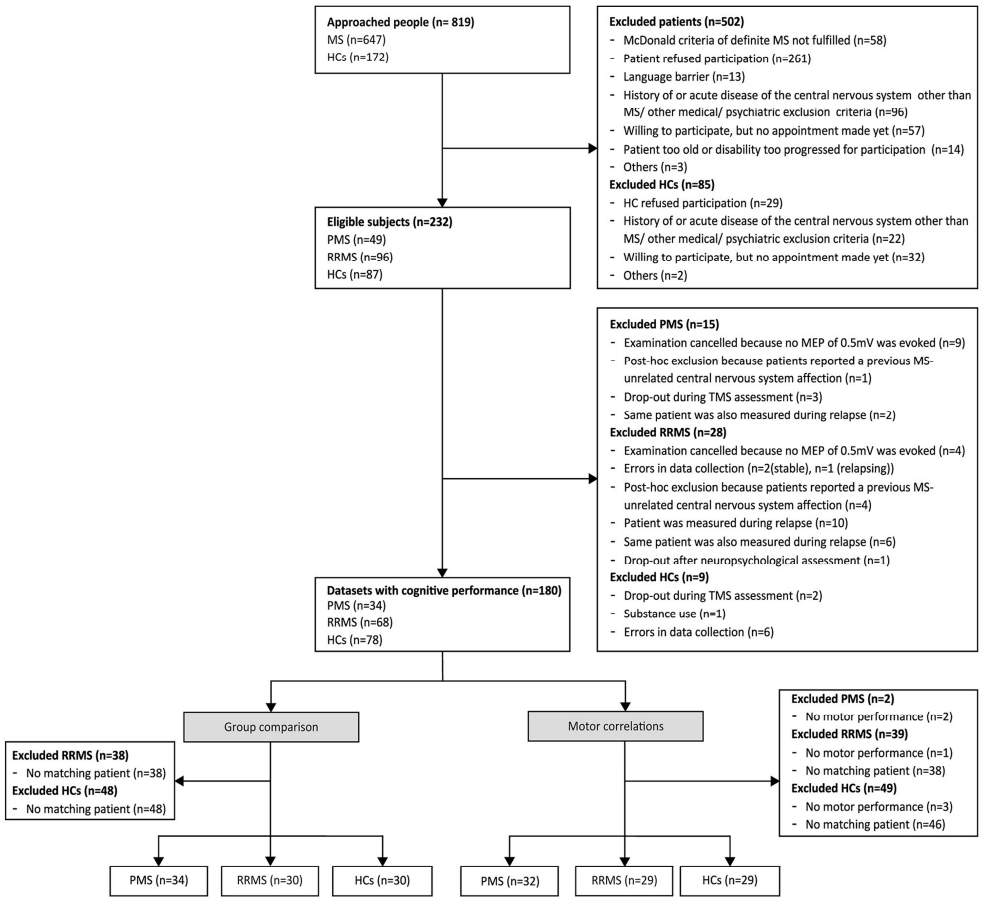

FIGURE 1
Flowchart of the enrollment of subjects. The flowchart presents the number of subjects at each step of the study. Since data on motor performance were missing in some subjects, slightly different subgroups were used to analyze the relationship between motor outcomes and cortical plasticity and to compare the degree of induced plasticity between disease types. HCs, healthy controls; MS, multiple sclerosis; MEP, motor-evoked potential; RRMS, relapsing-remitting multiple sclerosis; PMS, progressive multiple sclerosis (primary and secondary progressive multiple sclerosis); TMS, transcranial magnetic stimulation.

Lastly, we conducted exploratory analyses. In the first analysis, we divided the PMS group into SPMS and PPMS and repeated linear mixed-effect modeling with four instead of three groups to avoid systematic errors possibly evolving from the merger of both PMS groups. In the second and third analyses, we divided all patients with MS into two groups based on pyramidal tract integrity as measured by cortical latency. MEP latency is a measure of corticospinal conduction velocity and may be prolonged in patients with MS with pyramidal tract affection (28). Based on the clinical norms of the University Hospital Düsseldorf and to avoid misclassifications as "pathological," MEP latencies of <24.5 ms were considered normal. Spearman's rank correlation coefficients of ΔMEP with all functional readouts were calculated for both groups, following the same approach as described above. Uncorrected two-tailed p-values and Bonferroni–Holm-corrected (25, 26) p'-values are reported. Lastly, we compared the degree of induced plasticity between patients with pathological vs. normal cortical latency using linear mixed-effects models. The model computation followed the same procedures as described above. However, latency was not included as a covariate in the second analysis as patients were divided into two groups based on this variable.

3 Results

3.1 Demographic and clinical characteristics

Out of 819 people approached, a total of 34 patients with PMS (14 PPMS, 20 SPMS), as well as 30 matched patients with RRMS and 30 matched HCs were included (Figure 1). Demographic characteristics of each subgroup are presented in Table 1 and were compared between groups. As expected, patients with PMS were significantly more disabled than patients with RRMS and HCs, indicated by higher EDSS, longer cortical latency, worse performance in SDMT, BVMT-R, NHPT, and T25FW, and higher rates of unemployment. Furthermore, patients with PMS had higher active and resting motor thresholds than HCs and required higher stimulation intensity to evoke an MEP amplitude of ~0.5 mV compared to both patients with RRMS and HCs. Patients with RRMS required higher stimulation intensity to evoke an MEP amplitude of ~0.5 mV compared to HCs and performed worse on the NHPT as well as T25FW. The distribution of motor and cognitive fatigue was comparable between both patient groups, but, as expected, more patients described clinical levels of fatigue than HCs. Due to missing data in the T25FW and NHPT, 32 patients with PMS, 29 matched patients with RRMS, and 29 HCs were included in further analyses of the relationship between motor performance and cortical plasticity.

3.2 Differences in QPS-induced plasticity between patients with PMS and matched patients with RRMS and HCs

In all study groups, i.e., HCs, patients with RRMS, and patients with PMS, MEP amplitudes significantly increased after the QPS intervention. However, there was no difference in ΔMEP between groups (Table 2, Figures 2A, B). A significant main effect of cortical latency was revealed. Across all groups and both times of MEP measurement, longer latencies were associated with lower MEPs (Table 2).

To ensure that our statistical analyses were not fraught with systematic errors by merging PPMS and SPMS into one group of PMS, additional analyses were conducted separating patients with PPMS from patients with SPMS. The results did not reveal any significant differences in the degree of cortical plasticity (Supplementary Table 1, Supplementary Figure 1).

3.3 Association between functional readouts and QPS-induced plasticity

Concerning motor functions as measured by the T25FW and NHPT, ΔMEP correlated significantly with the time to complete the NHPT in patients with RRMS and HCs but not in patients with PMS. When controlling for multiple testing, significance was lost in HCs. No correlation was found between ΔMEP and the T25FW in either of the three groups. Despite the statistically significant correlation coefficients for the NHPT, data inspection revealed no clear linear relationship in either group for both measures of motor function (Figures 3A, B). We, therefore, modeled non-linear associations between both motor functions and ΔMEP, which did, however, not improve the model fit in either group.

Concerning cognitive functions, ΔMEP correlated significantly with the SDMT raw score in patients with RRMS and HCs but not in patients with PMS. Controlling for multiple testing, the association did not reach statistical significance in any group. ΔMEP also significantly correlated with the BVMT-R total score in patients with RRMS but missed statistical significance when controlling for multiple testing. No correlation was found for the BVMT-R in patients with PMS and HCs (Figures 3C, D).

Splitting the two patient groups by pyramidal tract integrity as measured by cortical latency, those patients with normal MEP latency showed significant correlations of ΔMEP with the NHPT, SDMT raw score, and the BVMT-R total score but not with the T25FW. The association remained statistically significant after the Bonferroni–Holm correction for the NHPT and SDMT raw score. Patients with pathological MEP latency did not show any correlation between ΔMEP and motor and cognitive readouts (Figures 3E–H).

Linear mixed-effects modeling revealed that QPS-induced plasticity was significantly reduced in patients with pathological compared to patients with normal cortical latency (Figures 2C, D, Table 3).

4 Discussion

This is the first study comparing QPS-induced plasticity of the motor cortex between HCs and different types of MS. Our study has two main findings. First, QPS-induced cortical plasticity did not differ between HCs and matched patients with RRMS and PMS. Second, we revealed intact corticospinal tract integrity as a prerequisite for the correlation between the degree of cortical plasticity and both motor and cognitive functions.

We found relevant associations between QPS-induced cortical plasticity and both motor and cognitive functions in patients with MS. However, this association was limited to cases in which MEP latencies, representing corticospinal conduction velocity (28), were normal. Importantly, exploratory analysis revealed that significantly higher degrees of plasticity were induced in these patients compared to patients with prolonged MEP latency. The relevance of structural integrity of the pyramidal tract for rTMS-induced cortical plasticity and learning abilities has already been shown in neurologically healthy subjects, suggesting rTMS to be valuable in identifying patients at risk of developing dementia (29). This is in line with our current results revealing pyramidal tract integrity as a requirement for the correlation of cortical plasticity and motor and cognitive function. Axonal cortical neurodegeneration with pyramidal tract affection may lead to lower synaptic density or activity and may therefore be relevant for plasticity impairment and functional deterioration in MS.

Importantly, the SDMT was conducted verbally, ensuring that no motor functions, apart from speech, were involved. Inaccuracies in the drawings of the BVMT-R due to motor dysfunctions (e.g., wriggly lines) were not considered in the scoring of the test. Therefore, motor dysfunction is unlikely to be responsible for

TABLE 1 Sample characteristics.

Characteristic	PMS ($n = 34$)	RRMS ($n = 30$)	HCs ($n = 30$)	p-value
Sex, *N* (%), female	16 (47)	16 (53)	16 (53)	0.90
Handedness, *N* (%), right[a]	32 (97)	27 (90)	27 (90)	0.55
Age, Md (IQR), years	52.5 (12)	48.5 (9)	53 (15)	0.25
Education, Md (IQR), years	15 (5)	16 (6)	17 (5)	0.21
Employment, *N* (%), yes	16 (47)	23 (77)	26 (87)	**0.002**[b]
AMT, Md (IQR), % MSO	45.5 (13)	44.5 (12)	39 (5)	**0.005**[c]
RMT, Md (IQR), % MSO	55.5 (15)	51.5 (20)	48 (7)	**0.007**[d]
MEP 0.5 mV, Md (IQR), % MSO	81 (35)	66.5 (32)	58 (12)	**<0.001**[e]
MEP latency, Md (IQR), ms	25.4 (6.4)	23.4 (2.5)	22.8 (2.8)	**<0.001**[f]
ΔPost-Pre MEP amplitude, Md (IQR), mV	0.2 (0.5)	0.5 (0.6)	0.5 (0.7)	0.27
BVMT-R				
Total learning score, Md (IQR)	19 (13)	23.5 (13)	28 (6)	**<0.001**[g]
z-score, Md (IQR)	−1.2 (2.4)	−0.1 (2.5)	0.81 (1.2)	**<0.001**[h]
SDMT				
Correct items, Md (IQR)	42.5 (16)	51.5 (24)	54.5 (20)	**<0.001**[i]
z-score, Md (IQR)	−1.2 (1.5)	−0.1 (1.9)	0.43 (2.0)	**<0.001**[i]
Nine-hole peg test				
Time to complete, Md (IQR), seconds	25.3 (9.9)	22.1 (5.4)	18.9 (2.2)	**<0.001**[j]
25-foot walk test				
Time to complete, Md (IQR), seconds	6.4 (4.2)	4.5 (1.8)	3.5 (1.1)	**<0.001**[k]
HADS, *N* (%), clinical				
Anxiety	1 (3)	5 (17)	0 (0)	**0.03**[l]
Depression	5 (15)	4 (13)	0 (0)	0.07
FSMC, *N* (%), mild/moderate/severe				
Motor	1 (3)/5 (15)/25 (74)	5 (17)/4 (13)/15 (50)	5 (17)/1 (3)/2 (7)	**<0.001**[m]
Cognitive	4 (12)/5 (15)/ 16 (47)	3 (10)/7 (23)/ 12 (40)	4 (13)/3 (10)/2 (7)	**<0.001**[m]
MS specific characteristics				
Disease duration, Md (IQR), years	12.2 (16)	13.5 (10)		0.86
EDSS, Md (IQR)[n]	5.0 (3.0)	2.0 (2.3)		**<0.001**
DMT at time of assessment, *N* (%)				0.35
None	6 (18)	5 (17)		
Natalizumab	4 (12)	7 (23)		
Ocrelizumab	21 (62)	12 (40)		
Fingolimod	1 (3)	1 (3)		
Cladribine	1 (3)	2 (7)		
Alemtuzumab	1 (3)	0 (0)		

(Continued)

TABLE 1 (Continued)

Characteristic	PMS ($n = 34$)	RRMS ($n = 30$)	HCs ($n = 30$)	p-value
Glatiramer acetate	0 (0)	2 (7)		
Dimethyl fumarate	0 (0)	1 (3)		

p-values < 0.05 (two-sided) were considered significant and are in boldface. HCs, healthy controls; PMS, patients with progressive multiple sclerosis; RRMS, patients with relapsing-remitting multiple sclerosis; Md, median; IQR, interquartile range; AMT, active motor threshold; RMT, resting motor threshold; MEP, motor-evoked potential; MEP 0.5 mV, stimulation intensity producing a reliable MEP of ~0.5 mV; MSO, maximal stimulator output; BVMT-R, Brief Visuospatial Memory Test-Revised; SDMT, Symbol Digit Modalities Test; HADS, Hospital Anxiety and Depression Scale; FSMC, Fatigue Scale of Motor and Cognition; EDSS, Expanded Disability Status Scale; DMT, disease-modifying therapy. [a]Missing data: $n = 1$ (PMS). [b]Uncorrected and Bonferroni–Holm-corrected pairwise chi-square tests revealed significant differences between HCs and PMS as well as PMS and RRMS. [c]Uncorrected Dunn's pairwise comparisons revealed significant differences between all groups. After the Bonferroni–Holm correction, only the difference between HCs and PMS remained significant. [d]Uncorrected and Bonferroni–Holm corrected Dunn's pairwise comparisons revealed a significant difference between HC and PMS. [e]Uncorrected and Bonferroni–Holm corrected Dunn's pairwise comparisons revealed significant differences between all groups. [f]Uncorrected and Bonferroni–Holm corrected Dunn's pairwise comparisons revealed significant differences between HCs and PMS as well as PMS and RRMS. Missing data: $n = 5$ (PMS: $n = 2$, RRMS: $n = 1$, HCs: $n = 2$). [g]Uncorrected and Bonferroni–Holm corrected Dunn's pairwise comparisons revealed significant differences between HCs and PMS as well as PMS and RRMS. [h]Uncorrected Dunn's pairwise comparisons revealed significant differences between all groups. After the Bonferroni–Holm adjustment, only the difference between HC and PMS remained significant. Calculation of z-scores based on the norms provided in the BVMT-R manual (19). [i]Uncorrected and Bonferroni–Holm corrected Dunn's pairwise comparisons revealed significant differences between HCs and PMS, as well as PMS and RRMS. Z-scores were calculated based on German norms (17). [j]Uncorrected and Bonferroni–Holm corrected Dunn's pairwise comparisons revealed significant differences between all groups. Missing data: $n = 5$ (2 PMS and 3 HCs). [k]Uncorrected and Bonferroni–Holm corrected Dunn's pairwise comparisons revealed significant differences between all groups. Patients, who were unable to walk the required distance ($n = 8$ [4 PMS, 4 RRMS]), were assigned the following time to complete: maximum time to complete (total MS Sample) + 1.645*standard deviation (total MS sample). Missing data: $n = 5$ (2 PMS, 3 HCs). [l]Uncorrected and Bonferroni–Holm corrected pairwise chi-square tests revealed no significant differences between any groups. Classification as "clinical" based on scores ≥ 11 (21). [m]Uncorrected and Bonferroni–Holm corrected pairwise chi-square tests revealed significant differences between HCs and PMS as well as HCs and RRMS. Classification (mild, moderate, and severe) based on cutoffs provided in the manual of the FSMC (20). [n]Missing data: $n = 1$ (PMS). MS, multiple sclerosis.

TABLE 2 Multivariable linear mixed-effects model of MEP amplitude over time in HCs, patients with RRMS, and patients with PMS.

	Fixed effects				Random effects
	β-coefficient (95% CI)	SE_b	t-value	p	SD
Intercept	+0.54(+0.49; +0.59)[a]	0.03	+21.28	**<0.0001**	
Pre-QPS	Reference				
Post-QPS	+0.51 (+0.33; +0.69)[a]	0.09	+5.73	**<0.0001**	
HCs	Reference				
RRMS	−0.03 (−0.09; +0.04)	0.03	−0.75	0.45	
PMS	−0.04 (−0.11; +0.04)	0.04	−1.02	0.31	
Age	−0.01 (−0.04; +0.02)	0.01	−0.77	0.45	
Latency	−0.04 (−0.07; −0.01)[a]	0.02	−2.45	**0.02**	
Post-QPS*RRMS	+0.02 (−0.23; +0.27)	0.18	+0.18	0.86	
Post-QPS*PMS	−0.11 (−∞; +0.09)	0.12	−0.91	0.18	
Age*Latency	+0.03 (−0.00; +0.06)	0.02	+1.92	0.06	
Subject*Pre-QPS					0.10
Subject*Post-QPS					0.51
Residual					0.08

Two-tailed 95% confidence intervals and p-values are displayed for all factors except for the primary variable of interest (post-QPS*PMS). For this factor, testing our hypothesis, that synaptic plasticity is reduced in patients with PMS, one-tailed 95% confidence intervals and p-values are reported. p-values of <0.05 are in boldface. t- and p-values are based on the asymptotic Wald test. Continuous variables (age, latency) centered at sample mean. R^2(conditional) = 0.97. R^2(marginal) = 0.32. Adjusted intraclass correlation coefficient = 0.95. QPS, quadripulse stimulation; HCs, healthy controls; RRMS, patients with relapsing-remitting multiple sclerosis; PMS, patients with progressive multiple sclerosis. [a]Indicates statistical significance. SE_b, standard error of β-coefficient.

the results. Nonetheless, we have conducted *post hoc* analyses comparing SDMT and BVMT-R results between patients with and without prolonged MEP latency. As expected, no significant differences were revealed between the groups. Thus, we conclude that pyramidal tract integrity could be an important factor to be controlled for in future plasticity studies in MS, e.g., by separately analyzing rTMS-induced plasticity in patients with normal and pathological cortical latency or even introducing pathological MEP latencies as an exclusion criterion.

Interestingly, only one of our motor outcomes, namely, the NHPT but not the T25FW, correlated with the degree of cortical plasticity. NHPT measures represented motor function from the left hemisphere to the right hand for which QPS-induced cortical plasticity of the left hemisphere corresponded to, while the results of the T25FW could have been influenced by other networks such as the cerebellar system and/or lesions in the spinal cord. In addition to age, performance in the T25FW has recently been associated with normalized deep gray matter volume, whereas the NHPT has been associated with normalized gray matter volume and cognitive performance (30). Thus, NHPT, BVMT-R, and SDMT may require more similar networks than T25FW, BVMT-R, and SDMT. In line with this,

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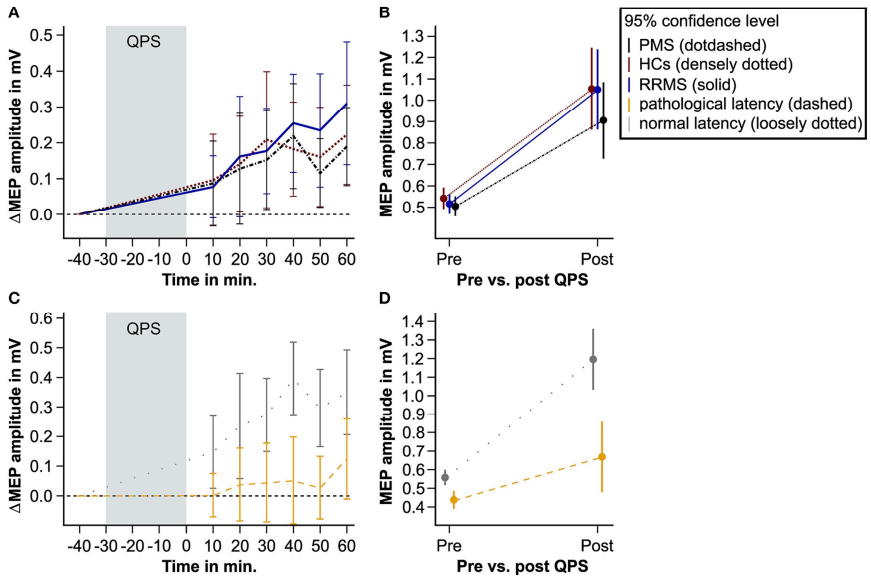

FIGURE 2
QPS-induced plasticity in patients with MS and matched HCs. This figure shows the level of QPS-induced plasticity in patients with PMS (black dot-dashed line), patients with RRMS (blue solid line), and HCs (red densely dotted line). Furthermore, it illustrates QPS-induced plasticity in patients with pathological (yellow dashed line) and normal MEP latency (gray loosely dotted line). **(A, C)** show the averaged difference between the pre- and post-QPS MEP amplitude in mV per time point and group. **(B, D)** illustrate the predicted MEP amplitude in mV based on the fixed effects of the linear mixed models pre- and post-QPS comparing HCs, PMS, and RRMS **(B)** and patients with pathological and normal MEP latency **(D)**. QPS, quadripulse stimulation; MS, multiple sclerosis; MEP, motor-evoked potential; HCs, healthy controls; RRMS, patients with relapsing-remitting multiple sclerosis; PMS, patients with progressive multiple sclerosis.

NHPT, BVMT-R, and SDMT were associated with cortical plasticity but not the T25FW.

We did not find cortical plasticity to be reduced in patients with PMS. This result contradicts the assumption that the progressive phase of the disease is characterized by insufficient compensatory reserve to balance out the negative consequences of inflammation and neurodegeneration (31). Furthermore, it is in contrast to an earlier study comparing TMS-induced cortical plasticity using iTBS and cTBS between patients with RRMS and PPMS (10). Patients with RRMS showed preserved plasticity, while it was absent in patients with PPMS after iTBS, which is supposed to induce LTP-like plasticity. Interestingly, cortical plasticity still turned out to be altered in patients with RRMS since cTBS, which originally had been supposed to induce LTD-like plasticity, led to a reversal of plasticity and induced LTP-like effects (10). The authors suggested that platelet-derived growth factor (PDGF) may play a substantial role in LTP induction in patients with MS. Although we did not measure PDGF levels in the cerebrospinal fluid in our study, other reasons may account for the different results between these two studies. In the earlier study, MEP latency, RMT, and AMT were also significantly higher in patients with PPMS, suggesting relevant pyramidal tract affection in this group. Therefore, given the importance of pyramidal tract integrity revealed in the present study, the difference in induced cortical plasticity revealed in the earlier study (10) may have been driven by pyramidal tract affection rather than the type of MS. Furthermore, higher rates of variability have been described for iTBS and cTBS and verified also in direct comparison to QPS recently (6, 8, 12). Considering the low sample size of patients with PPMS in the previous study ($n = 12$) (10), alterations of induced plasticity in this group may have been an unsystematic result of high variability of previously used protocols rather than a systematic difference between disease types. Moreover, although recent TMS work postulated that loss of inhibition may be particularly important in SPMS (32), it has been shown that excitatory glutamatergic circuits may play a key role in MS pathology (33–35). In contrast to iTBS and cTBS, which influence both excitatory and inhibitory networks, QPS is supposed to selectively modulate excitatory glutamatergic cortical networks (5). Thus, the QPS protocol may induce LTP more efficiently in patients with MS and therefore may have yielded different results than the iTBS and cTBS protocols. Future studies should compare the effects of different rTMS protocols in patients with MS intraindividually to reveal the strengths and limitations of each protocol and thus increase the quality of future investigations of plasticity in MS.

Recruitment of patients with PMS for rTMS research may have some pitfalls, the most difficult being the relatively low prevalence rate compared to RRMS. Moreover, patients with PMS are typically

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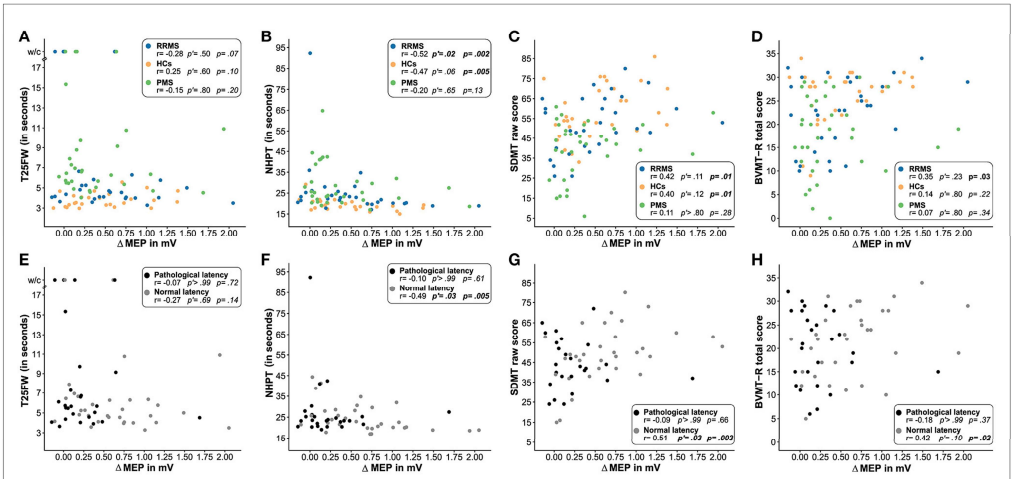

FIGURE 3
Association of the degree of induced plasticity with functional readouts. This figure illustrates the association of the degree of induced plasticity (ΔMEP) with the T25FW **(A, E)**, NHPT **(B, F)**, SDMT **(C, G)**, and BVMT-R **(D, H)** in patients with RRMS (blue), PMS (green), and HCs (orange) and in patients with pathological (gray) and normal (black) cortical latency. Uncorrected one-tailed *p*-values and Bonferroni–Holm-corrected *p*′-values are reported for **(A–D)**. For **(E–H)**, uncorrected two-tailed *p*-values and Bonferroni–Holm-corrected *p*′-values are reported. *P*-values of < 0.05 are displayed in bold face. HCs, healthy controls; RRMS, patients with relapsing-remitting multiple sclerosis; PMS, patients with progressive multiple sclerosis; T25FW, timed 25-foot walk test; w/c, wheelchair; NHPT, nine-hole peg test; BVMT-R, Brief Visuospatial Memory Test-Revised; SDMT, Symbol Digit Modalities Test; MEP, motor-evoked potential; ΔMEP, difference between the maximum of the six mean MEP amplitude after repetitive quadripulse stimulation and the MEP amplitude before stimulation.

TABLE 3 Multivariable linear mixed-effects model of MEP amplitude over time in patients with normal vs. patients with pathological cortical latency.

	Fixed effects				Random effects
	β-coefficient (95% CI)	SE_b	*t*-value	p	SD
Intercept	+0.56 (+0.52; +0.60)[a]	0.02	+26.82	**<0.0001**	
Pre-QPS	Reference				
Post-QPS	+0.64 (+0.49; +0.79)[a]	0.08	+8.36	**<0.0001**	
Normal cortical latency	Reference				
Pathological cortical latency	−0.12 (−0.18; −0.06)[a]	0.03	−3.75	**<0.001**	
Post-QPS*Pathological cortical latency	−0.41 (−0.64; −0.17)[a]	0.12	−3.49	**<0.001**	
Subject*Pre-QPS					0.09
Subject*Post-QPS					0.49
Residual					0.08

Two-tailed 95% confidence intervals, and *p*-values are displayed. *p*-values of <0.05 are in boldface. *t*- and *p*-values are based on the asymptotic Wald test. R^2(conditional) = 0.97. R^2(marginal) = 0.42. Adjusted intraclass correlation coefficient = 0.95. QPS, quadripulse stimulation. [a]Indicates statistical significance. SE_b, standard error of β-coefficient.

more severely impaired (13, 14), impeding participation in studies with extensive protocols due to exhaustion or mobility issues. Despite these challenges, we included a sample that was almost three times as big ($n = 34$ vs. $n = 12$) as in the previous study by Mori et al. (10). We decided to summarize patients with PPMS and SPMS to one group of PMS to increase the statistical power since the clinical disease and pathophysiology appear to be similar (36). However, lower levels of white matter lesions and inflammation have been described for PPMS (31, 36), and it is still an open debate whether this disease subtype represents the same or a distinct disease entity. Therefore, we conducted an exploratory analysis, in which we compared QPS-induced plasticity in patients with PPMS and SPMS separately to matched HCs. However, we did not find any differences across groups, supporting

the legitimacy of our approach to summarize the two progressive disease types.

Our study is not without limitations. Due to the cross-sectional design, no conclusions regarding the clinical relevance for disease progression can be drawn. Furthermore, the lack of imaging data prevents us from analyzing the impact of (sub)cortical lesions. Thus, we cannot rule out that MEP latencies may have not only been prolonged due to damaged pyramidal tracts but also due to abnormalities in the motor cortex. In addition, MEP latency may have been influenced by the participant's height and age. Patients with MS received different disease-modifying therapies and symptomatic medications, which potentially have impacted cortical excitability. Even though no systematic evaluation of the effects of different treatments on cortical excitability exists to the best of our knowledge, stabilizing effects of disease-modifying drugs on cortical excitability over time have been suggested in patients with PMS (37). Due to high variations in the stimulation protocols, target muscles, and study populations, different numbers of averaged trials to achieve reliable MEP assessments have been recommended (38–41). We chose to average 12 MEPs at each time of assessment to maintain a concise protocol and minimize participant fatigue. However, this number of average trials is at the lower end of the recommendations, and we might have improved the reliability of our findings by increasing the number of averaged MEPs. Lastly, baseline MEP amplitude was controlled to be ~0.5 mV in all patients. However, this amplitude could have been distributed at varying places on the recruitment curve for the different subjects (42), potentially causing ceiling effects in patients with impaired corticospinal tract integrity (43). Furthermore, QPS may have affected MEP size differentially depending on the stimulation intensity relative to the recruitment curve (5, 44).

Although overall cortical plasticity between RRMS and PMS was comparable on the group level, i.e., the degree of QPS-induced plasticity did not differ between them, it is plausible that there were disparities in the proportion of patients with corticospinal dysfunction and the extent of such dysfunction between groups. This is supported by the fact that PMS patients had longer MEP latencies compared to RRMS patients. In accordance with this, we identified associations between QPS-induced plasticity and behavioral outcomes only among patients with normal MEP latency, primarily those with RRMS, but not among patients with prolonged MEP latency, primarily those with PMS. In patients with prolonged MEP latency, it is conceivable that damage to the corticospinal tract exerted a more pronounced influence on QPS-induced plasticity, potentially overshadowing other associations, such as those between QPS-induced plasticity and behavioral measures. However, due to the exploratory character of this discovery in our study, we can only speculate about its neuropathological underpinnings, which warrant further investigation.

Despite these limitations, our study supports the notion of pyramidal tract integrity being of more relevance for QPS-induced cortical plasticity in MS and related functional significance than the amount of progression.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by ethical committee of the medical faculty of the Heinrich Heine University Düsseldorf. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft. PA: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft. A-SS: Data curation, Formal analysis, Investigation, Writing – review & editing. LS: Investigation, Writing – review & editing. SN: Investigation, Writing – review & editing. CH: Investigation, Writing – review & editing. SM: Resources, Writing – review & editing. AS: Resources, Writing – review & editing. I-KP: Conceptualization, Funding acquisition, Methodology, Writing – original draft. SG: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft.

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Conflict of interest

PA received compensation for serving on Scientific Advisory Boards for Allergan, Celgene, Janssen Cilag, Ipsen, Merck, Merz Pharmaceuticals, Novartis, and Biogen; he received speaker honoraria and travel support from Novartis, Teva, Biogen, Celgene, Merz Pharmaceuticals, Ipsen, Allergan, Bayer Healthcare, Esai, UCB; Roche; he received research support from Novartis, Allergan, Biogen, Celgene, Teva, Merz Pharmaceuticals, Ipsen, and Roche. CH has been serving as a consultant for Univar and has received honoraria for lecturing and travel expenses/speaking honoraria from Abbott and Alexion, and research support from Abbott. SM has received honoraria for lecturing and travel expenses for attending meetings from Almirall, Amicus Therapeutics Germany, Bayer Health Care, Biogen, Celgene, Diamed, Genzyme, MedDay Pharmaceuticals, Merck Serono, Novartis, Novo Nordisk, ONO Pharma, Roche, SanofiAventis, Chugai Pharma, QuintilesIMS, and Teva, and research funding from the German Ministry for Education and Research (BMBF), the Deutsche Forschungsgemeinschaft, the Else Kröner Fresenius Foundation, the German Academic Exchange Service, the Hertie Foundation, the Interdisciplinary Center for Clinical Studies (IZKF) Muenster, the German Foundation for Neurology, Almirall, Amicus Therapeutics, Germany, Biogen, Diamed, Fresenius Medical Care, Genzyme, Merck Serono, Novartis, ONO Pharma, Roche, and Teva. AS has received lecture fees from Abbott, Novartis, and Kyowa Kirin, and has been serving as a consultant for Abbott, Zambon, and Medtronic Inc. He received royalties from Georg Thieme Verlag. He is a government employee and receives through his institution funding for his research from the German Research Council, Abbott, and the Brunhilde Moll Foundation. I-KP received honoraria for speaking at scientific meetings, serving at scientific advisory boards, and consulting activities from Almirall, Bayer Pharma, Biogen, BMS, Celgene, Sanofi-Genzyme, Janssen, Merck, Novartis, Roche, and Teva. She received research support from the German MS Society, Celgene, Novartis, Roche, and Teva. SG received honoraria and/or travel expenses from Abbott Medical, Abbvie, Boston Scientific, Medtronic, Rogue Research, and UCB, consulting fees from Zambon, and research support from Abbott and Hilde-Ulrichs Stiftung.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1266225/full#supplementary-material

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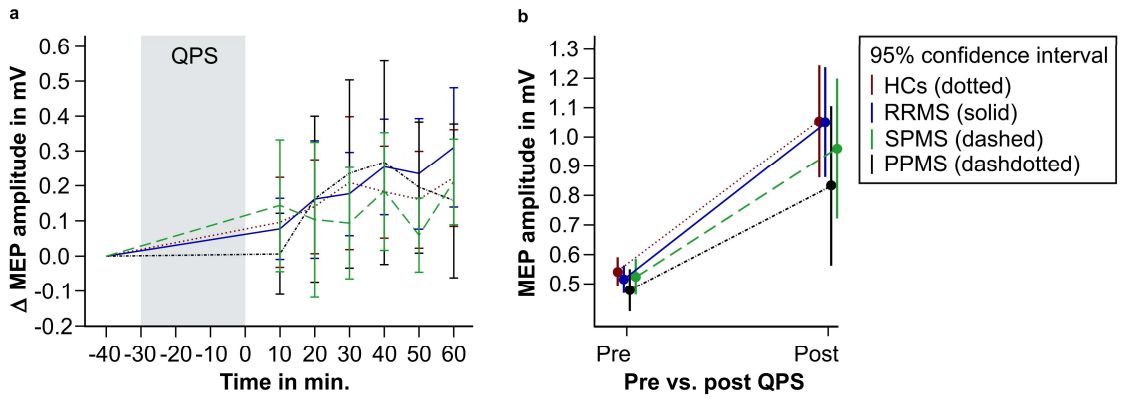
Supplementary Material

1 Supplementary Table 1. Multivariable linear mixed-effects model of MEP amplitude over time in HCs, patients with RRMS, PPMS and SPMS

Fixed Effects					**Random Effects**
	β-coefficient (95% CI)	*SE_b*	*t-value*	*p*	*SD*
Intercept	+0.54 (0.49; 0.59)[a]	0.03	+21.21	**<.0001**	
Pre QPS	Reference				
Post QPS	+0.51 (+0.33; +0.69)[a]	0.09	+5.71	**<.0001**	
HCs	Reference				
RRMS patients	-0.03 (-0.09; +0.04)	0.03	-0.73	.47	
PPMS patients	-0.06 (-0.15; +0.03)	0.05	-1.38	.17	
SPMS patients	-0.02 (-0.10; +0.07)	0.04	-0.40	.69	
Age	-0.01 (-0.04; +0.02)	0.01	-0.54	.59	
Latency	-0.04 (-0.07; -0.01)[a]	0.02	-2.46	**.02**	
Post QPS*RRMS	+0.02 (-0.23; +0.27)	0.13	+0.18	.86	
Post QPS*PPMS	-0.16 (-0.47; +0.15)	0.16	-1.01	.32	
Post QPS*SPMS	-0.08 (-0.36; -0.08)	0.14	-0.53	.60	
Age*Latency	+0.03 (+0.00; +0.07)[a]	0.02	+2.08	**.04**	
Subject*Pre QPS					0.10
Subject*Post QPS					0.51
Residual					0.08

Note. Two-tailed 95% confidence intervals and *p*-values are displayed. *p*-values <.05 are in boldface. *t*- and *p*-values are based on asymptotic Wald test. Continuous variables (age, latency) centered at sample mean. R^2(conditional)=0.97. R^2(marginal)=0.32. Adjusted Intraclass Correlation Coefficient=0.95.
QPS= Quadripulse stimulation. HCs=Healthy Controls. RRMS= Relapsing remitting Multiple Sclerosis. PPMS= Primary progressive Multiple Sclerosis; SPMS= Secondary progressive Multiple Sclerosis. SE_b= Standard error of the β-coefficient.
[a] indicates statistical significance.



Supplementary Figure 1. QPS-induced plasticity separately for patients with PPMS and SPMS compared to matched pwRRMS and HCs.

This figure shows the level of QPS-induced plasticity in patients with PPMS (black dashdotted line), SPMS (green dashed line), RRMS (blue solid line), and HCs (red dotted line). Part A shows the averaged difference between the pre and post QPS MEP amplitude per time point in all groups. Part B illustrates the predicted MEP amplitude in mV based on the fixed effects of the linear mixed models pre and post QPS. QPS=Quadripulse stimulation; MEP=Motor evoked potential; HCs=Healthy Controls; RRMS=Relapsing-remitting multiple sclerosis; SPMS=Secondary progressive multiple sclerosis; PPMS= Primary progressive multiple sclerosis

Study 4 – Prognostic value of synaptic plasticity for disease progression

Balloff, C., Janssen, L.K., S., Hartmann, C. J., Meuth, S.G., Schnitzler, A., Penner, I.-K.,[8] & Albrecht, P.[8] (2024). *Predictive value of synaptic plasticity for functional decline in patients with multiple sclerosis* [Manuscript submitted for publication].

Conceptualization & methodology: I defined the research question and analysis plan in consultation with P. Albrecht and I.-K. Penner based on the experimental design, which was created by P. Albrecht, and I.-K. Penner.

Investigation & project administration: I supervised recruitment of participants and data collection. I recruited participants and independently performed neuropsychological tests and rTMS. I was also responsible for data curation. Project administration was performed by me, P. Albrecht, and I.-K. Penner. P. Albrecht and C.J. Hartmann conducted neurological assessments. L.K. Janssen recruited participants, conducted neuropsychological tests under supervision and provided support in data curation. P. Albrecht contributed to the recruitment of participants.

Formal analysis: I conducted the statistical analyses with contributions by L.K. Janssen and reviewed them for correctness.

Resources: I provided funding for the open access fee. P. Albrecht provided funding for this study. Further resources were provided by S.G. Meuth and A. Schnitzler.

Manuscript: I wrote the initial manuscript, which included all steps from comprehensive literature research to final formulation. I created the figures and tables with contributions of L.K. Janssen. All authors critically reviewed the manuscript. I.-K. Penner, and P. Albrecht additionally contributed to the original draft.

[8] Shared last authorship.

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Predictive value of synaptic plasticity for functional decline in patients with multiple sclerosis

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Keywords: synaptic plasticity, multiple sclerosis, repetitive transcranial magnetic stimulation, quadripulse stimulation, functional decline, disease progression

Abstract

Background: Previous research suggested that quadripulse (QPS)-induced synaptic plasticity is associated with both cognitive and motor function in patients with multiple sclerosis (MS) and does not appear to be reduced compared to healthy controls (HCs).

Objective: This study aimed to explore the relationship between the degree of QPS-induced plasticity and clinically significant decline in motor and cognitive functions over time. We hypothesized that MS patients experiencing functional decline would exhibit lower levels of baseline plasticity compared to those without decline.

Methods: QPS-induced plasticity was evaluated in 80 MS patients (56 with relapsing-remitting MS and 24 with progressive MS), and 69 age-, sex-, and education-matched HCs. Cognitive and motor functions, as well as overall disability status were evaluated annually over a median follow-up period of two years. Clinically meaningful change thresholds were predefined for each outcome measure. Linear mixed-effects models, Cox proportional hazard models, logistic regression, and receiver-operating characteristic analysis were applied to analyse the relationship between baseline plasticity and clinical progression in the Symbol Digit Modalities Test, Brief Visuospatial Memory Test Revised (BVMT-R), nine-hole peg test (NHPT), timed 25-foot walk test, and Expanded Disability Status Scale.

Results: Overall, the patient cohort showed no clinically relevant change in any functional outcome over time. Variability in performance was observed across time points in both patients and HCs. MS patients who experienced clinically relevant decline in manual dexterity and/or visuospatial learning

and memory had significantly lower levels of synaptic plasticity at baseline compared to those without such decline (NHPT: β=-0.25, p=.02; BVMT-R: β=-0.50, p=.005). Receiver-operating characteristic analysis underscored the predictive utility of baseline synaptic plasticity in discerning between patients experiencing functional decline and those maintaining stability only for visuospatial learning and memory (area under the curve=0.85).

Conclusions: Our study suggests that QPS-induced plasticity could be linked to clinically relevant functional decline in patients with MS. However, to solidify these findings, longer follow-up periods are warranted, especially in cohorts with higher prevalences of functional decline. Additionally, the variability in cognitive performance in both patients with MS and HCs underscores the importance of conducting further research on reliable change based on neuropsychological tests.

1 Introduction

Multiple Sclerosis (MS) is a neurological disease, characterized by inflammatory, demyelinating lesions and neurodegeneration (1), leading to a wide range of motor, sensory and cognitive impairments (2). For most patients (~85%), the disease manifests as relapsing-remitting MS (RRMS), which is characterized by sudden episodes of new or exacerbated neurological symptoms alternating with periods of symptom remission and clinical stability (3). Over time, frequency of symptom remission decreases, and a majority of untreated RRMS patients (>80%) progress to secondary progressive MS (SPMS) within 25 years (4). SPMS involves a progressive worsening of symptoms with or without acute exacerbations (5). In contrast to RRMS and SPMS, individuals with primary progressive MS (PPMS), do not experience acute exacerbations. Instead, symptoms increase gradually starting from disease onset (6). PPMS affects approximately 10-20% of MS patients (7).

The mechanisms contributing to disability accumulation in MS are still not fully understood (8) and are discussed controversially. Despite efforts to identify biomarkers indicative of disease activity and progression, their clinical utility at the individual level is constrained (9). This challenge has been called attention to by the long-standing recognition of the 'clinico-radiological paradox', which underscores the discrepancies between observed lesion burden in brain imaging and clinical symptom presentation (10). In addition to brain and cognitive reserve (11), this paradox might be attributed to neuroplasticity, i.e. the brain's ability to adapt and reorganize (12, 13). Neuroplasticity may compensate for structural damage, albeit to a diminishing extent as the disease advances. Although neuroplasticity involves several aspects, reorganization at the synaptic level through the reinforcement or weakening of synapses, known as long-term potentiation (LTP) or long-term depression (LTD), respectively, is a key component of synaptic plasticity (14).

One emerging avenue of investigating synaptic plasticity non-invasively involves the application of quadripulse stimulation (QPS), a transcranial magnetic stimulation (TMS) protocol known for its ability to induce both LTP and LTD in healthy subjects (15). We have previously investigated the functional relevance of LTP-like plasticity induced by QPS in patients with MS cross-sectionally and revealed that levels of QPS-induced plasticity correlate with cognitive and motor function among individuals with intact pyramidal tract integrity (16). Importantly, the level of LTP-like plasticity was not reduced in patients of all disease types compared to healthy controls (HCs) (16, 17).

Previous studies regarding the clinical relevance of LTP-like plasticity for MS disease progression have relied solely on cross-sectional designs and revealed conflicting results. One comparison of LTP-like plasticity between RRMS and PPMS patients suggested a potential association between diminished levels of plasticity and disease progression (18). In contrast, our attempt to replicate this finding using QPS did not confirm it, but instead indicated comparable levels of LTP-like plasticity across both groups (16). Furthermore, another study found that enhanced synaptic plasticity after four

weeks of oral D-aspartate treatment was not associated with improvements in clinical outcomes (19). To date, longitudinal studies regarding the clinical relevance of LTP-like plasticity for MS disease progression are lacking to the best of our knowledge. However, research on the transition to dementia in individuals with memory impairment has proposed that LTP-like plasticity could potentially serve as a predictive biomarker for clinical progression (20).

In summary, cross-sectional studies have yielded inconclusive results regarding the clinical relevance of LTP-like plasticity for MS disease progression. Longitudinal studies are warranted to comprehensively explore this aspect. Therefore, the objective of this study was to examine the relationship between the degree of LTP-like plasticity at baseline and disease progression up to five years after QPS assessment in patients with MS. Drawing from previous research that has suggested a potential association between QPS-induced plasticity and clinical outcomes (16, 17), and considering the promising results in the field of dementia (20), we hypothesized that patients with lower baseline plasticity levels would exhibit greater disease progression compared to those with higher plasticity levels.

2 Materials and Methods

Subjects

Patients diagnosed with definite MS according to the 2017 revised McDonald criteria (21) were enrolled in the study, along with age-, sex-, and education-matched healthy controls (HCs). Monocentric recruitment of patients occurred at the neurological clinic of the University Hospital Düsseldorf, Germany, from May 2018 to October 2022. HCs were recruited as a convenience sample from an internal database of interested HCs, friends, and family members of faculty members of the University Hospital Düsseldorf. All participants were invited for annual follow-ups for up to five years after baseline assessment.

Participants with at least one follow-up after baseline assessment were included. Exclusion criteria comprised a history of neurological or psychiatric disorders other than MS and remitted depressive episodes at baseline or follow-up. Additional exclusion criteria included contraindications for TMS and substance or alcohol abuse, which were assessed through a TMS safety screening questionnaire (22). At baseline and each follow-up, patients were required to be relapse free for ≥ 30 days and appointments were postponed in case patients did not fulfill this requirement.

The study received ethical approval from the ethical committee of the medical faculty of Heinrich Heine University Düsseldorf (study-number 2018-16), and informed written consent was obtained from all participants before study participation. The study adhered to the principles of the Declaration of Helsinki.

Experimental designs and procedure

The experimental design of this longitudinal study is summarized in Figure 1. At baseline, data were assessed according to the procedures described in our previous studies (17, 16, 23). To summarize, baseline assessment was divided into three parts: 1) neurological examination, 2) neuropsychological examination consisting of the Symbol Digit Modalities Test (SDMT) (24) as a measure of information processing speed, the Brief Visuospatial Memory Test Revised (BVMT-R) (25) as a measure of visuospatial short-term memory and learning, the Hospital Anxiety and Depression Scale (HADS) (26) as a measure of anxiety and depression, and the Fatigue Scale for Motor and Cognitive Functions (FSMC) (27) as a measure of trait fatigue, and 3) assessment of QPS-induced synaptic plasticity using a facilitatory protocol (interstimulus interval 5ms, QPS-5) (28). QPS-induced

plasticity was operationalized by the change in MEP amplitudes recorded at the right first dorsal interosseous muscle following QPS-5 of the contralateral motor cortex. MEP amplitude prior to QPS-5 was adjusted to be ~0.5 mV in all participants. After 30 minutes of QPS-5, MEP responses evoked by the same pre-interventional stimulation intensity were recorded for a total of 60 minutes at the same muscle. At each time of assessment, it was intended to average 12 MEPs. However, due to artifacts or voluntary muscle activity, certain MEPs had to be excluded from the calculation of the averaged MEP amplitude, resulting in a median of 11 averaged MEPs for each time point and subject.

Assessment of motor function involved the nine-hole peg test (NHPT) to evaluate manual dexterity and fine motor skills, while the timed 25-foot walk (T25FWT) was used as a measure of walking ability (29). Additionally, the EDSS was used to gauge the extent of disability attributed to MS.

Our objective was to carry out in-person assessments during follow-ups. However, unforeseen circumstances (e.g. COVID-19 pandemic) occasionally resulted in participants being unavailable for in-person appointments. In such instances, remote follow-up assessments using a video conferencing tool were conducted, consisting of a structured interview, followed by cognitive tests. Patient-reported outcome measures (HADS, FSMC) were filled out by the participant immediately after the video conference. No assessments of motor functions were conducted during remote follow-ups (see supplementary material for details on remote assessment). Independent of assessment mode (remote vs. in-person), alternative forms of the BVMT-R were used at each follow-up to minimize practice effects.

Definition of clinically meaningful change

The objective of this study was to investigate the predictive value of synaptic plasticity for clinically meaningful change in cognition, motor function, and global disability, as measured by EDSS. Since these outcomes are subject to day-to-day fluctuations, cut-off values to discriminate clinically meaningful change from expected measurement variability were required.

In line with common research practice, clinically meaningful change in the EDSS was defined as a change of ≥ 1 point for baseline scores ≤ 5.5 and a change of ≥ 0.5 point for baseline scores ≥ 6.0 (30, 31). For the SDMT, a change of ≥ 8 raw score points was considered clinically significant, since it has recently been demonstrated that this cut-off is more reliable than the previously used cut-off of ≥4 raw score points (32, 33). Regarding reliable change on the BVMT-R, we incorporated a cut-off of ≥8 points in the total learning trials, which has been presented at the 28th Congress of the European Committee for Treatment and Research in Multiple Sclerosis by the BICAMS initiative (34). For both the T25FWT and the NHPT, the well-established cut-off of a change of ≥20% was considered clinically significant (35, 31, 36).

Statistical analyses

Sample size was determined based on the number of eligible MS patients and matched HCs, since this is the first study investigating rTMS-induced plasticity as a prognostic marker of disease progression in patients with MS. No imputation was performed to address missing data, except for participants who were unable to complete the T25FWT due to disability. In line with our previous study (16), imputation for patients unable to perform the T25FWT was based on the following formula:

$$Time\ in\ ms = maximum\ time\ within\ the\ total\ MS\ cohort\ across\ all\ times\ of\ assessment + 1.645 * SD\ within\ the\ total\ MS\ cohort\ across\ all\ times\ of\ assessment$$

Chi-square test was used to compare the number of subjects with clinically relevant decline/improvement between patients with MS and HCs. The absolute changes in each outcome from BL to latest FU were compared among groups using Kruskal-Wallis test. Significant omnibus tests were followed by Dunn's test to ascertain the specific group difference(s).

Linear mixed-effects models (LMEM) were employed for each functional outcome (SDMT, BVMT-R, NHPT, T25FWT, EDSS) to compare the level of baseline plasticity between patients with clinically relevant decline at latest FU and those without. Consistent with our prior cross-sectional investigations of QPS-induced plasticity in patients with MS (17, 23, 16) and given the good reproducibility and standardization of this approach (28), baseline synaptic plasticity was defined based on MEP amplitude changes from pre to post QPS (ΔMEP). The model consisted of fixed effects of the intervention (pre/post-QPS) and group classification (relevant decline/no relevant decline), as well as their interaction (QPS*group) and was estimated using the "restricted maximum likelihood method". The subject-specific variability in response to QPS was considered by a random slope for the QPS intervention (pre/post-QPS). In the context of our hypothesis of reduced baseline plasticity in patients with clinically relevant decline at latest FU, the interaction (QPS*group) was of primary interest for each functional outcome, as significant interactions would suggest significant differences in the extent of plasticity among the respective groups. Significance of this effect was tested based on one-tailed confidence intervals and *p*-values of the QPS*group factor. Confidence intervals and *p*-values of all other factors were based on two-tailed analysis.

Consistent with our previous studies (17, 16, 23), additional factors such as age, depression, anxiety, fatigue, and MEP latency, along with their interactions with the QPS intervention, were separately introduced into the model. Furthermore, disease duration, EDSS at baseline, and time since baseline were introduced to account for different clinical baseline characteristics and different follow-up times. However, baseline EDSS was not introduced to the model comparing QPS-induced plasticity between patients with and without EDSS worsening, as baseline EDSS is a strong predictor of EDSS worsening with lower baseline EDSS being associated with more change over time (37, 38).

Each of these more complex models was compared against the simplest model through likelihood-ratio tests or, in instances of missing data in covariates, through the Akaike information criterion. Collinearity was assessed using the variance inflation factor (cutoff value of ≥5). The best fitting model is presented.

In addition to the LMEM, time to event analysis using Cox proportional hazard models correcting for age at baseline and sex, were conducted to compare the probability of clinically meaningful change in each functional outcome between patients with high and low baseline plasticity, which was defined based on a median split of ΔMEP. Event rates for both groups (high vs. low plasticity) were estimated using Kaplan-Meier analysis. Additionally, receiver-operating characteristic analysis and logistic regression were conducted to evaluate the ability of baseline plasticity to discriminate between patients with and without clinically relevant decline in the functional outcomes. Logistic regression was performed using plasticity at baseline (ΔMEP) and time since baseline as continuous predictors of functional decline (yes vs. no) in each outcome (SDMT, BVMT-R, NHPT, T25FWT, EDSS). To interpret the results of the receiver-operating characteristic analysis, the area under the curve was calculated.

The nlme package in R Studio (version 2023.12.1+402 for windows) were used to conduct LMEM. All other analyses were conducted using IBM SPSS Statistics (version 29.0.1.0 for windows).

3 Results

Out of 96 patients and 75 HCs included at baseline assessment, 56 patients with RRMS, 24 patients with PMS (14 SPMS, 10 PPMS) and 69 HCs completed at least one follow-up assessment (see Figure 2).

Neurological and neuropsychological trajectories

Table 1 shows the descriptive and clinical characteristics of all patients and HCs at baseline and latest follow-up. Performance on the SDMT, BVMT-R, NHPT, T25FWT, and EDSS at baseline and each FU are presented in Figure 3, illustrating substantial fluctuations across time in both patients as well as HCs. Figure 4 illustrates the individual absolute change in each functional measure from BL to latest FU as well as a summary on the group level. While the median absolute change differed significantly from zero for some outcomes and groups (SDMT: all groups except SPMS, NHPT: HCs, BVMT-R: RRMS), on the group level, none of these changes surpassed the defined thresholds for clinically meaningful change in any outcome. Comparing the absolute change per outcome between groups (HCs, RRMS, PPMS, SPMS) revealed a significant difference in the absolute SDMT change between patients with PPMS and both RRMS (Bonferroni-corrected p=.009) and HCs (Bonferroni-corrected p=.03). None of the other outcomes differed significantly between groups.

Analyzing individual data, n=35 (69%) patients with MS compared to n=16 (31%) HCs experienced decline in any of the functional outcomes (p=.008, Φ=.22). The EDSS exhibited the highest incidence of clinically relevant decline among the outcome measures, with n=19 (24%) patients experiencing such decline. However, a comparable number of patients also demonstrated clinically relevant EDSS improvement (n=15, 19%). In the T25FWT, n=9 (12%) patients presented with clinically meaningful decline compared to n=3 (4%) patients with clinically relevant improvement. Both the NHPT and the SDMT showed n=7 (9%) patients with clinically meaningful decline, but more patients improved in the SDMT (n=15, 19%) than in the NHPT (n=4, 5%). Only n=3 (4%) exceeded the cognitive decline cut-off on the BVMT-R, whereas n=7 (9%) demonstrated clinical improvement.

Examining HCs, n=7 (11%) experienced decline on the T25FWT, compared to n=4 (6%), n=5 (7%), and n=0 on the SDMT, BVMT-R, and NHPT, respectively. Clinically relevant improvement was observed in n=13 (19%) on the SDMT, n=10 (14%) on the BVMT-R, n=1 (2%) on the NHPT, and n=2 (3%) on the T25FWT. Comparing the number of events of clinically relevant decline between HCs and patients with MS, significantly more patients than HCs experienced clinically relevant decline in the NHPT (p=.016, Φ =.21). No significant differences in the number of events were detected for all other outcomes.

Predictive value of QPS-induced plasticity for functional decline

Figure 5 illustrates the increase of MEP amplitude following QPS in patients with clinically relevant decline in the functional outcome measures compared to those without clinically relevant decline. Increase in MEP amplitude following QPS was significantly lower in patients with clinically meaningful decline in the NHPT (β=-0.25, p=.02) and BVMT-R (β=-0.50, p=.005) compared to clinically stable patients, as indicated by a significant QPS*group interaction. No significant differences were detected for progression in the SDMT (β=+0.39, p=.09), T25FWT (β=-0.18, p=.07), and EDSS (β=+0.11, p=.23). The final LMEMs for each functional outcome are presented in *Supplementary Table 1-5.*

Receiver-operating characteristic analysis revealed high accuracy of ΔMEP at baseline to differentiate between patients with and without clinically relevant decline in BVMT-R at latest

follow-up (area under the curve=0.853). For all other outcomes, ΔMEP at baseline could not discriminate between patients with vs. without clinically relevant decline.

Cox-Proportional Hazard Models and logistic regression did not reveal associations between the degree of baseline plasticity and clinically relevant decline in any functional measure. Kaplan-Meier curves with the results of the Cox-Proportional Hazard models are presented in *Supplementary Figure 1*. *Supplementary Figure 2* illustrates the receiver operating characteristic curves and the odds ratios (including 95% confidence intervals) of clinically meaningful decline are presented in *Supplementary Table 6.*

4 Discussion

To the best of our knowledge, this is the first study investigating the prognostic value of QPS-induced plasticity in patients with MS. In this cohort of MS patients, only a small number of patients exhibited clinically meaningful declines in the SDMT, BVMT-R, NHPT, T25FWT, and EDSS during a median observational period of two years. Importantly, the number of patients demonstrating clinically meaningful change did not significantly differ from HCs, except for the NHPT. However, comparing patients with clinically relevant functional decline in the BVMT-R and NHPT using LMEM revealed significantly lower levels of baseline plasticity in patients with functional decline in these measures. ROC analysis indicated predictive utility of baseline synaptic plasticity in discerning between patients experiencing functional decline and those maintaining stability only for the BVMT-R. Other statistical approaches, such as Cox proportional Hazard models and logistic regression, did not reveal significant differences between groups and no association between baseline plasticity and functional decline was observed for the T25FWT, SDMT and EDSS.

Our results confirm previous studies showing that both cognitive and physical disability progression occur slowly in patients with MS and assessment of cognitive function appears to be volatile. A previous study examining a five-year follow-up period reported cognitive decline in 28% of MS patients, with a higher incidence observed among PMS compared to RRMS patients (39). Even after 10 years of follow-up, clinically relevant changes in cognitive or physical disability at the group level have been reported to be scarce, with only 24% of patients displaying cognitive decline (40). Another study also spanning a 10-year follow-up period reported a 10% increase in the overall proportion of MS patients exhibiting cognitive impairment. Notably, the authors reported a dynamic pattern of cognitive function, where some patients demonstrated cognitive improvement in specific domains, while others experienced impairment in different cognitive domains during follow-up compared to baseline assessments (41). However, contrasting reports of higher rates of cognitive decline—50% and 62% over periods of 6 and 7 years, respectively—have also been documented (42, 43). Consequently, the literature presents considerable variations in rates of cognitive decline over time, influenced by factors such as patients' demographics, definitions of cognitive decline, applied neuropsychological test batteries, frequency of assessments, and duration of follow-up. Another explanation for the low rate of clinically relevant deterioration in our cohort may be the high percentage of patients on high efficacy therapy. This is a common phenomenon in cohorts of patients recruited at tertiary referral centers.

Importantly, we report a higher proportion of patients exhibiting improvement in cognitive tests compared to those demonstrating decline. This aligns with recent data from a study involving RRMS patients treated with subcutaneous daclizumab or intramuscular interferon beta-1a, reporting more frequent improvement in the Paced Auditory Serial Addition Test and SDMT than decline 144 weeks post-baseline assessment. This phenomenon might be attributed to practice effects in the previous study as participants were tested every six months (44). However, we also did not observe a

significant difference in decline between MS patients and HCs in the SDMT or the BVMT-R despite less frequent testing and the use of alternative forms for the BVMT-R at each assessment. Conversely, clinically relevant decline was more prevalent than improvement in motor function outcomes for patients with MS, and significantly more patients with MS experienced decline in the NHPT than HCs. These findings underscore the necessity for further research on reliable change indexes in neuropsychological tests, especially in the context of annual assessments.

Notable fluctuations have also been described for repeated assessment using the EDSS. In a randomized controlled trial, 21% of patients receiving a placebo exhibited significant improvement on the EDSS during a 5-year follow-up, while 25% experienced significant worsening. Conversely, patients receiving immunotherapy with cladribine demonstrated improvement in 18% and worsening in 16% of cases (45). In our cohort, we observed a similar trend, with 24% of patients experiencing a clinically relevant decline in EDSS scores compared to 19% showing improvement. These findings align with existing literature and highlight concerns regarding the reliability and sensitivity of the EDSS to detect meaningful changes over time (30). Notably, one criticism of the EDSS is its emphasis on ambulation issues for scores ≥ 6, often overlooking other important functional deficits (46).

The LMEM revealed significant differences in baseline plasticity between patients with and without meaningful decline only for the BVMT-R and NHPT, but not for the SDMT, T25FWT, and the EDSS. Regarding the SDMT, this observation may stem from its lack of specificity, as noted in previous research (47). Despite its sensitivity to detect cognitive impairment in patients with MS, the SDMT lacks specificity, since a patient's performance on this test does not only rely on cognitive processing speed but also involves other cognitive aspects such as working memory, paired-associate learning, and visual scanning, albeit to a lesser extent (48). In contrast, the BVMT-R serves as a sensitive measure of learning and memory, less prone to confounding other cognitive functions. However, it may be marginally influenced by manual impairments owing to the drawing component of the test (47). This may explain the congruence in LMEM results between the NHPT and BVMT-R. Two out of three patients exhibiting decline on the BVMT-R also demonstrated decline on the NHPT, which assessed manual dexterity of the dominant hand in all cases. Nonetheless, the differential results for the SDMT and BVMT-R contrast with our previous cross-sectional results, which revealed significant correlations between QPS-induced plasticity and performance on both the BVMT-R and SDMT in patients with RRMS and those with normal cortical latency (17, 16). Importantly, the limited number of patients showing decline on the BVMT-R warrants consideration as well.

Regarding changes in the T25FWT, recent research affirmed the clinical utility of a 20% change cutoff (44). The observation that a higher proportion of patients experienced clinically meaningful decline compared to improvement (12% vs. 4%) in our cohort further supports the validity of this cutoff. The absence of an association between baseline plasticity and clinical decline on the T25FWT aligns with our earlier discovery of no cross-sectional correlation with QPS-induced plasticity (16). As discussed previously, this may be explained by the test's susceptibility to influences from spinal and cerebellar lesions, aspects potentially not fully captured by QPS.

Importantly, this study exclusively examined QPS-5-induced plasticity, evaluating LTP-like synaptic plasticity, which is a rapid-onset mechanism of neuroplasticity (15, 49), However, focusing solely on these rapid-onset mechanisms might not comprehensively capture all facets of neuroplasticity. Other parts of plasticity, e.g. LTD-like plasticity may also be relevant, considering the potential involvement of inhibitory circuits in MS (50). Rather than isolating either of these two synaptic plasticity types, their interplay could be crucial. In fact, our previous study indicated that stabilizing

metaplasticity during relapses might be more pivotal than LTP-like plasticity itself in preventing motor disability (16).

Furthermore, we neither controlled for cognitive reserve (51), nor therapeutic interventions between follow-ups. In general, high levels of education at baseline (median years of education =16 years) indicated high levels of cognitive reserve in our patient cohort, potentially explaining the low prevalence of cognitive decline. However, cognitive rehabilitation, potentially taking place between follow-ups, may have influenced our outcome measures as well. Emerging evidence indicates that patients with MS, particularly those with RRMS, the predominant subgroup in our cohort, may benefit from such interventions (52). Given the intricate and dynamic nature of plasticity, changes in plasticity may have occurred between baseline and follow-up, possibly in both directions (enhanced and diminished plasticity).

Expanding on our earlier finding underscoring the importance of evaluating synaptic plasticity within the context of corticospinal tract integrity among MS patients (16), our goal was to conduct subgroup analyses to assess the predictive value of QPS-induced plasticity in patients with pathological latency compared to those with normal latency. Due to the low number of events, i.e. patients experiencing functional decline, this was not feasible. Additionally, given the relatively small number of patients with PMS, and especially PPMS, we decided against performing subtype specific analyses.

Despite these limitations, we present a large cohort of MS patients with longitudinal clinical follow-ups and baseline plasticity assessment using QPS-5. Moreover, the inclusion of closely matched HCs allows for a comparison of changes observed between MS patients and HCs. We conclude that LTP-like synaptic plasticity may be of functional relevance in patients with MS and that more research is needed to identify and better define reliable change in cognitive performance in these patients.

5 Figure legends

Figure 1. Summary of study design

This figure summarizes the experimental design. Baseline assessment consisted of a short neuropsychological test battery, a neurological examination and transcranial magnetic stimulation, including QPS. Details are described in our previous publications (17, 16, 23).

EDSS= Expanded Disability Status Scale; SDMT= Symbol Digit Modalities Test; BVMT-R= Brief Visuospatial Memory Test – Revised; NHPT= Nine-hole peg test; T25FWT= Timed 25-foot walk test; PROMs= Patient reported outcome measures; QPS=Quadripulse stimulation; AMT= Active motor threshold

Figure 2. Longitudinal cohort flowchart

This figure summarizes the number of participants throughout the study.

RRMS= Patients with relapsing-remitting multiple sclerosis; PMS= Patients with progressive multiple sclerosis; HCs= Healthy controls; yr= Year; yrs= Years

Figure 3. Individual data for each assessment and timepoint

This figure displays the raw data for each assessment and timepoint per subject for the SDMT (A), BVMT-R (B), NHPT (C), T25FWT (D), and EDSS (E). Lines connect data from the same subject. EDSS was missing for one patient at baseline. Patients with primary or secondary progressive

multiple sclerosis are displayed in the same color (red), but different symbols (square for PPMS, cross for SPMS).

SDMT= Symbol Digit Modalities Test; BVMT-R= Brief Visuospatial Memory Test – Revised; NHPT= Nine-hole peg test; T25FWT= Timed 25-foot walk test; w/c= wheelchair; EDSS= Expanded Disability Status Scale; PPMS= Patients with primary progressive multiple sclerosis; SPMS= Patients with secondary progressive multiple sclerosis; RRMS= Patients with relapsing-remitting multiple sclerosis; HCs= Healthy controls

Figure 4. Absolute changes in functional measures from baseline to latest follow-up by group

This figure displays the absolute change in each functional measure from baseline to latest follow-up by group. Total score changes for SDMT, BVMT-R, and EDSS are included, while changes in completion time in seconds are provided for NHPT and T25FWT. Extreme values, defined as scores falling below the first quartile minus three times the interquartile range (1st quartile – 3*IQR) or exceeding the third quartile plus three times the interquartile range (3rd quartile + 3*IQR), are denoted by asterisks, alongside their precise numerical values. Patients with primary or secondary progressive multiple sclerosis are displayed in the same color (red), but different symbols (square for PPMS, cross for SPMS).

IQR= Interquartile range; SDMT= Symbol Digit Modalities Test; BVMT-R= Brief Visuospatial Memory Test – Revised; NHPT= Nine-hole peg test; T25FWT= Timed 25-foot walk test; EDSS= Expanded Disability Status Scale; PPMS= Patients with primary progressive multiple sclerosis; SPMS= Patients with secondary progressive multiple sclerosis; RRMS= Patients with relapsing-remitting multiple sclerosis; HCs= Healthy controls; PMS= Patients with primary or secondary progressive multiple sclerosis

Figure 5. QPS-induced plasticity at baseline in patients with different clinical outcomes at latest follow-up

This figure displays the level of QPS-induced plasticity at baseline in patients with clinically relevant decline at latest follow-up (solid gray line) compared to patients without clinically relevant decline (dashed black line) in each functional outcome (A= BVMT-R, B= SDMT, C= NHPT, D= T25FWT, E= EDSS).

QPS= Quadripulse stimulation; BVMT-R= Brief Visuospatial Memory Test – Revised; SDMT= Symbol Digit Modalities Test; NHPT= Nine-hole peg test; T25FWT= Timed 25-foot walk test; EDSS= Expanded Disability Status Scale.

6 Tables

Table 1. Sample characteristics at baseline and latest follow-up

Characteristic	RRMS		PMS		HCs	
	BL (n=56)	FU (n=52)	BL (n=24)	FU (n=28)	BL (n=69)	FU (n=69)
Completed follow-ups, Md (IQR)[a]	2 (2)		2 (2)		2 (2)	
Time since baseline, Md (IQR), months	29 (20)		27 (17)		26 (22)	
Sex, N (%), female[b]	37 (66)	35 (67)	14 (58)	16 (57)	43 (62)	
Age, Md (IQR), years	39 (17)	40 (16)	54 (12)	56 (12)	36 (31)	37 (30)

Education, Md (IQR), years	15 (4)	16 (4)	15 (5)	15 (5)	16 (3)	17 (4)
MEP latency, Md (IQR), ms[c]	22.69 (4)		25.06 (7)		22.56 (2)	
ΔPost-Pre MEP amplitude,	0.48		0.21		0.47	
Md (IQR), mV	(0.51)		(0.67)		(0.58)	
HADS, N (%), clinical[d]						
Anxiety	9 (16)	7 (14)	1 (4)	2 (7)	1 (1)	0
Depression	6 (11)	4 (8)	2 (8)	4 (14)	0	0
FSMC, N (%), moderate or severe[e]						
Motor	26 (46)	26 (50)	20 (83)	25 (89)	4 (6)	6 (9)
Cognitive	23 (41)	22 (42)	14 (58)	17 (61)	7 (10)	5 (7)
Disease duration, Md (IQR), years	9 (12)	13 (12)	8 (16)	12 (17)		
EDSS, Md (IQR)[f]	1.5 (3)	2 (3)	5 (4)	5.75 (3)		
DMT at time of assessment, N (%)[g]						
None	9 (16)	5 (10)	4 (17)	4 (14)		
Group 1	3 (5)	4 (8)	0	0		
Group 2	4 (7)	6 (12)	2 (8)	5 (18)		
Group 3	40 (71)	37 (71)	18 (75)	19 (68)		

Note. Median and interquartile range are displayed for metric variables due to non-normal distribution in at least one group at one time of assessment. n=4 RRMS patients converted to secondary progressive multiple sclerosis at latest follow-up. HCs= Healthy controls. Md=Median. IQR=Interquartile range. MEP= Motor evoked potential. HADS=Hospital Anxiety and Depression Scale. FSMC= Fatigue Scale of Motor and Cognition. EDSS= Expanded Disability Status Scale. DMT= Disease-modifying therapy. BL= Baseline. FU= Follow-up.

[a] Remote assessment at latest follow-up: RRMS: n=2, HCs: n=2.

[b] Sex defined as sex assigned at birth.

[c] Missing data: RRMS: n=4, PMS: n=3, HCs: n=19.

[d] Defined as scores ≥ 11 (26). Missing data: HCs BL: n=3.

[e] Defined based on cut-offs provided in the FSMC manual (27). Missing data: HCs BL: n=3.

[f] Missing data: RRMS n=1 at baseline

[g] Groups based on the current guidelines in Germany (S2k-Leitlinie) (53): Group 1=beta-interferone, dimethyl fumarate, teriflunomide, glatirameroide, group 2= cladribine, s1p-receptor modulators, group3= alemtuzumab, CD20-antibodies, natalizumab. One patient (SPMS) was on intravenous immunoglobulin therapy at BL and FU, which is currently not approved as a DMT in patients with MS in Germany. However, this patient presented with contraindications for immunotherapy and gammaglobuline deficiency. This treatment was assigned to group 2.

7 Conflict of Interest

C. Balloff has received speaker honoraria and travel expenses from Roche and speaker honoraria from Synaptikon GmbH.

L.K. Janßen has received an individual funding granted by the research committee of the medical faculty of the Heinrich Heine University Düsseldorf for her doctoral thesis (October 2021 - March 2022).

C.J. Hartmann has been serving as consultant for Univar and has received honoraria for lecturing and travel expenses/speaking honoraria from Abbott and Alexion, and research support from Abbott.

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A. Schnitzler has received lecture fees from Abbott, Novartis, Kyowa Kirin, and has been serving as a consultant for Abbott, Zambon, Medtronic Inc. He received royalties from Georg Thieme Verlag. He is a government employee and receives through his institution funding for his research from the German Research Council, Abbott, and the Brunhilde Moll Foundation.
I.-K. Penner received honoraria for speaking at scientific meetings, serving at scientific advisory boards, and consulting activities from Almirall, Biogen, BMS, Celgene, Sanofi-Genzyme, Janssen, Merck, Novartis, Roche, and Teva. She received research support from the German MS Society, Celgene, Novartis, Roche, and Teva.
P. Albrecht received compensation for serving on Scientific Advisory Boards for Allergan, Abbvie, Biogen, Bristol Myers Squibb, Celgene, Janssen Cilag, Ipsen, Merck, Merz Pharmaceuticals, Novartis, Biogen; he received speaker honoraria and travel support from Novartis, Teva, Biogen, Bristol Myers Squibb, Celgene, Merz Pharmaceuticals, Ipsen, Allergan, Bayer Healthcare, Esai, UCB, Lilly, Roche, Sanofi; he received research support from German Research Association (DFG), Bristol Myers Squibb, Novartis, Allergan, Abbvie, Biogen, Celgene, Teva, Merz Pharmaceuticals, Ipsen, and Roche.

8 Author Contributions

C. Balloff: Conceptualization, investigation, data curation, methodology, project administration, formal analysis, visualization, writing - original draft. L.K. Janßen: Investigation, formal analysis, visualization, writing - review & editing. C.J. Hartmann: Investigation, writing - review & editing. S.G. Meuth: Resources, writing - review & editing. A. Schnitzler: Resources, writing - review & editing. I.-K. Penner: Conceptualization, funding acquisition, methodology, writing - original draft. P. Albrecht: Conceptualization, investigation, funding acquisition, methodology, writing - original draft.

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11 Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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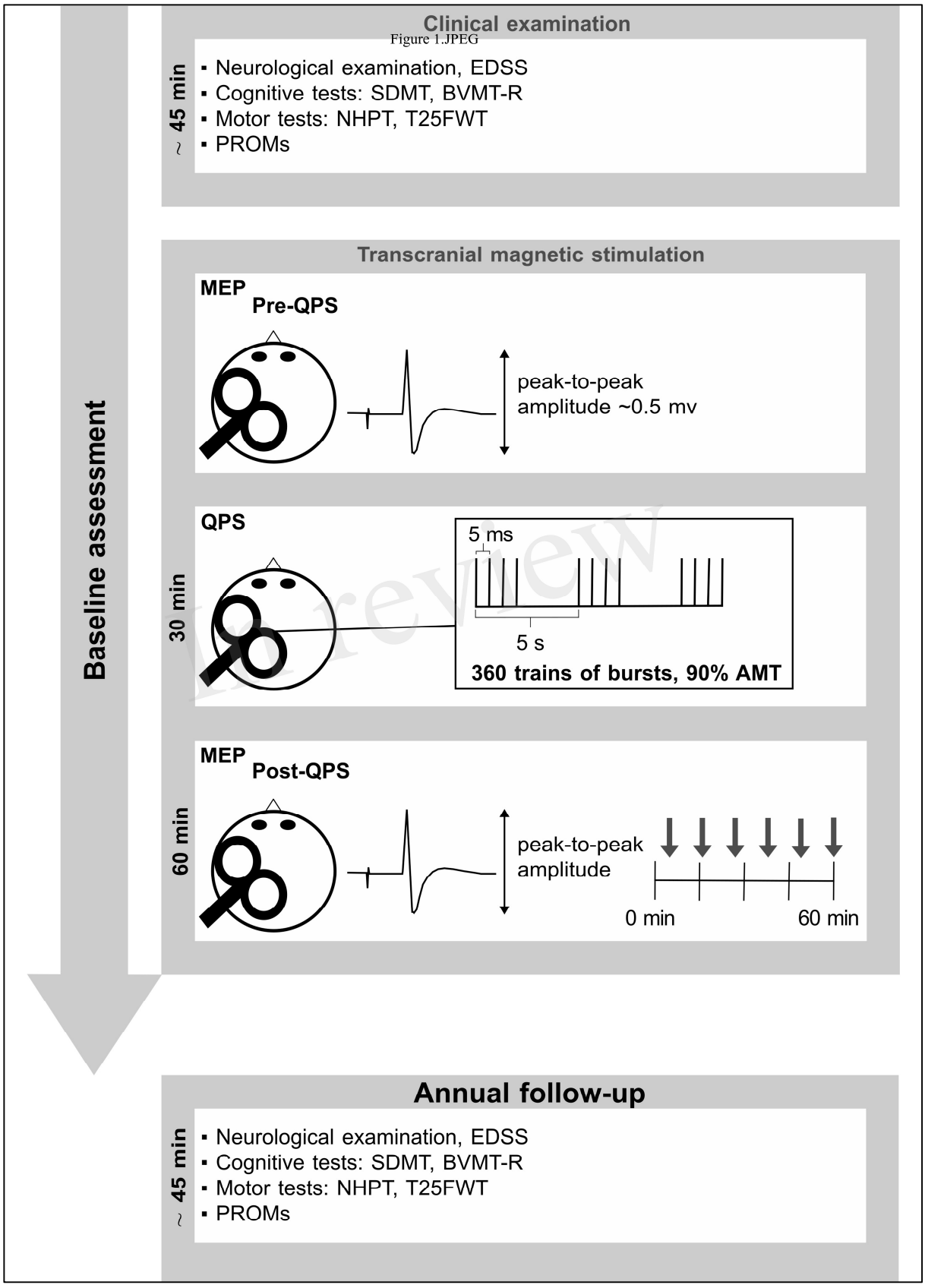
Figure 1.JPEG



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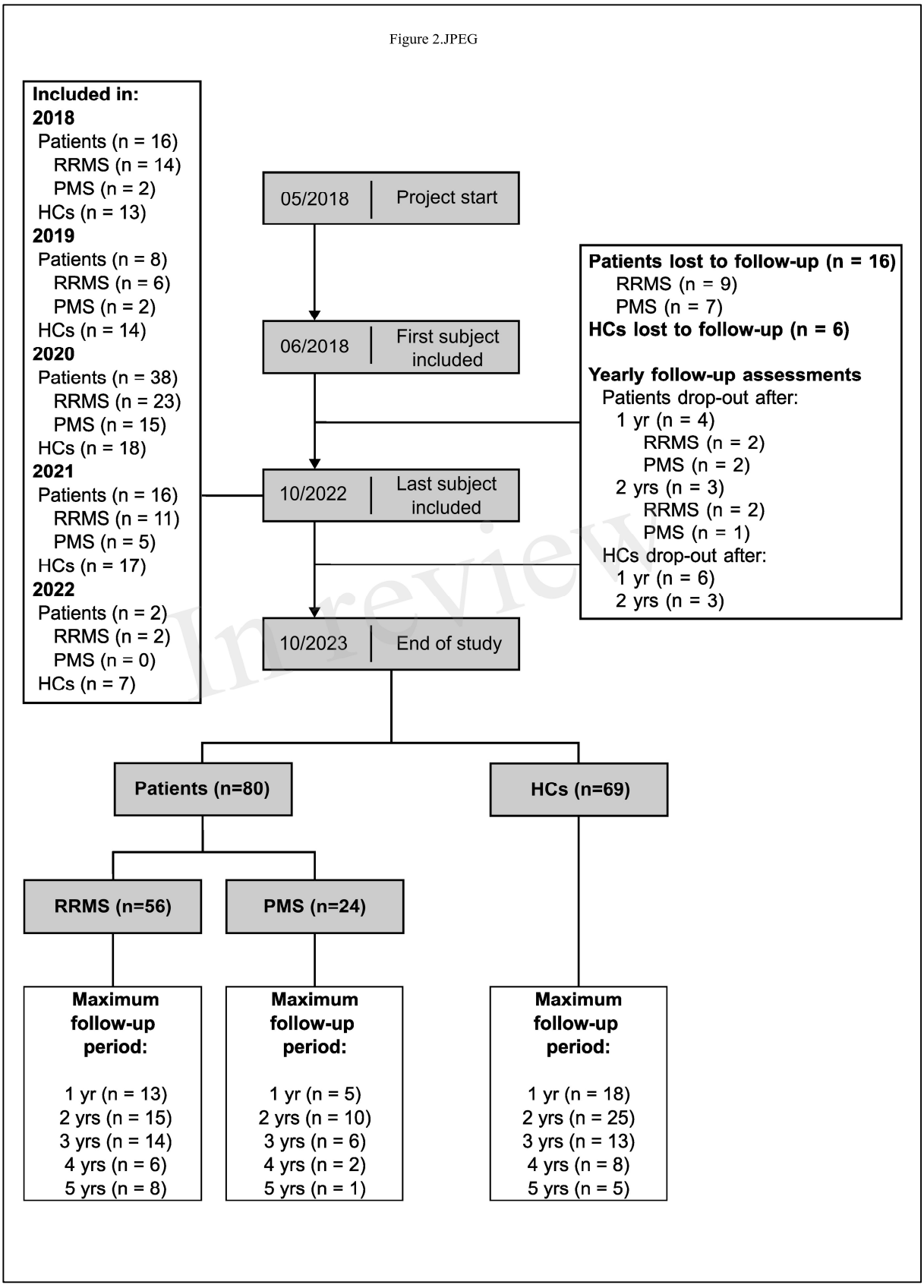
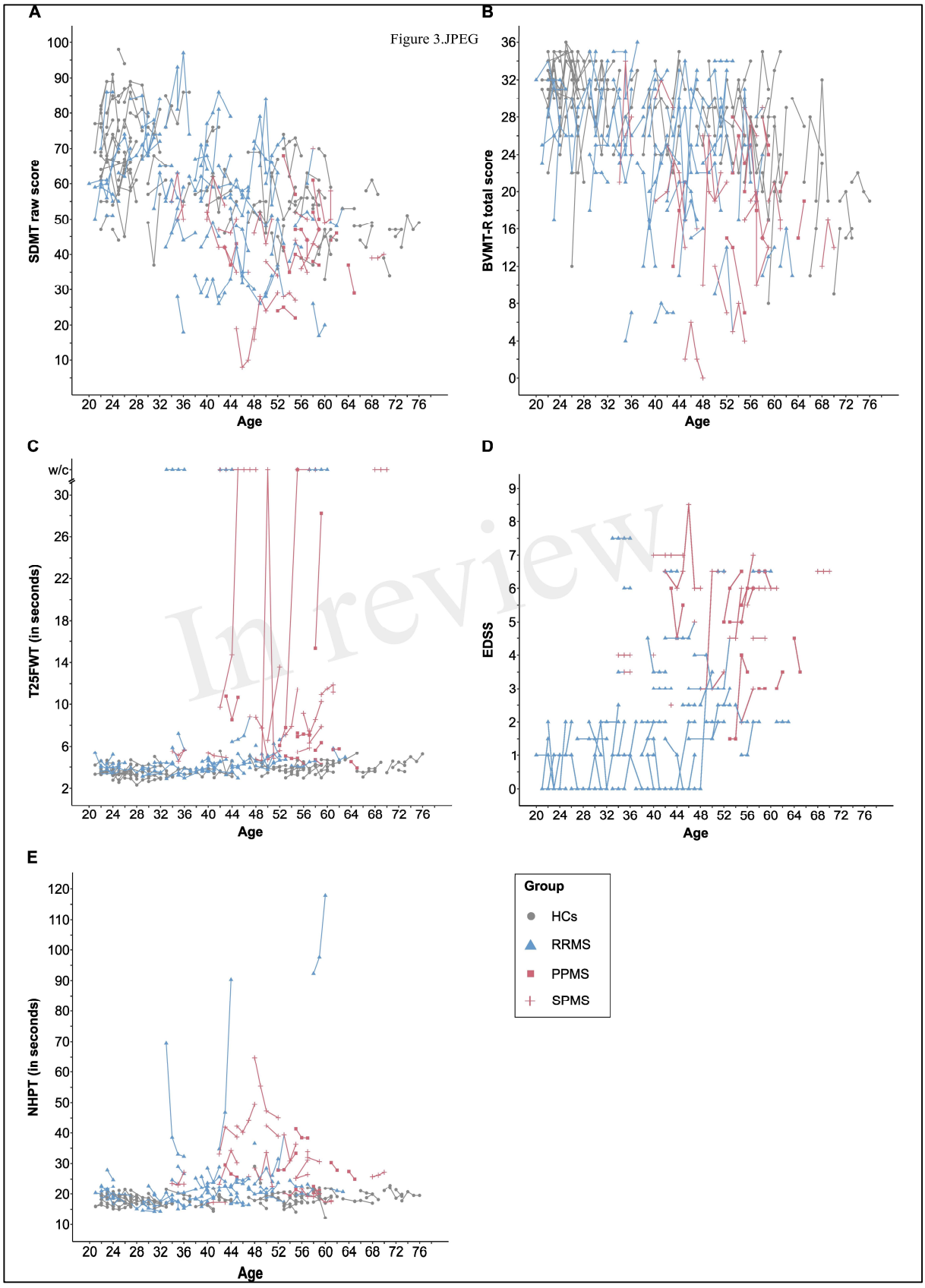



A
Figure 3.JPEG
SDMT raw score
Age
B
BVMT-R total score
Age
C
w/c
T25FWT (in seconds)
Age
D
EDSS
Age
E
NHPT (in seconds)
Age
Group
HCs
RRMS
PPMS
SPMS

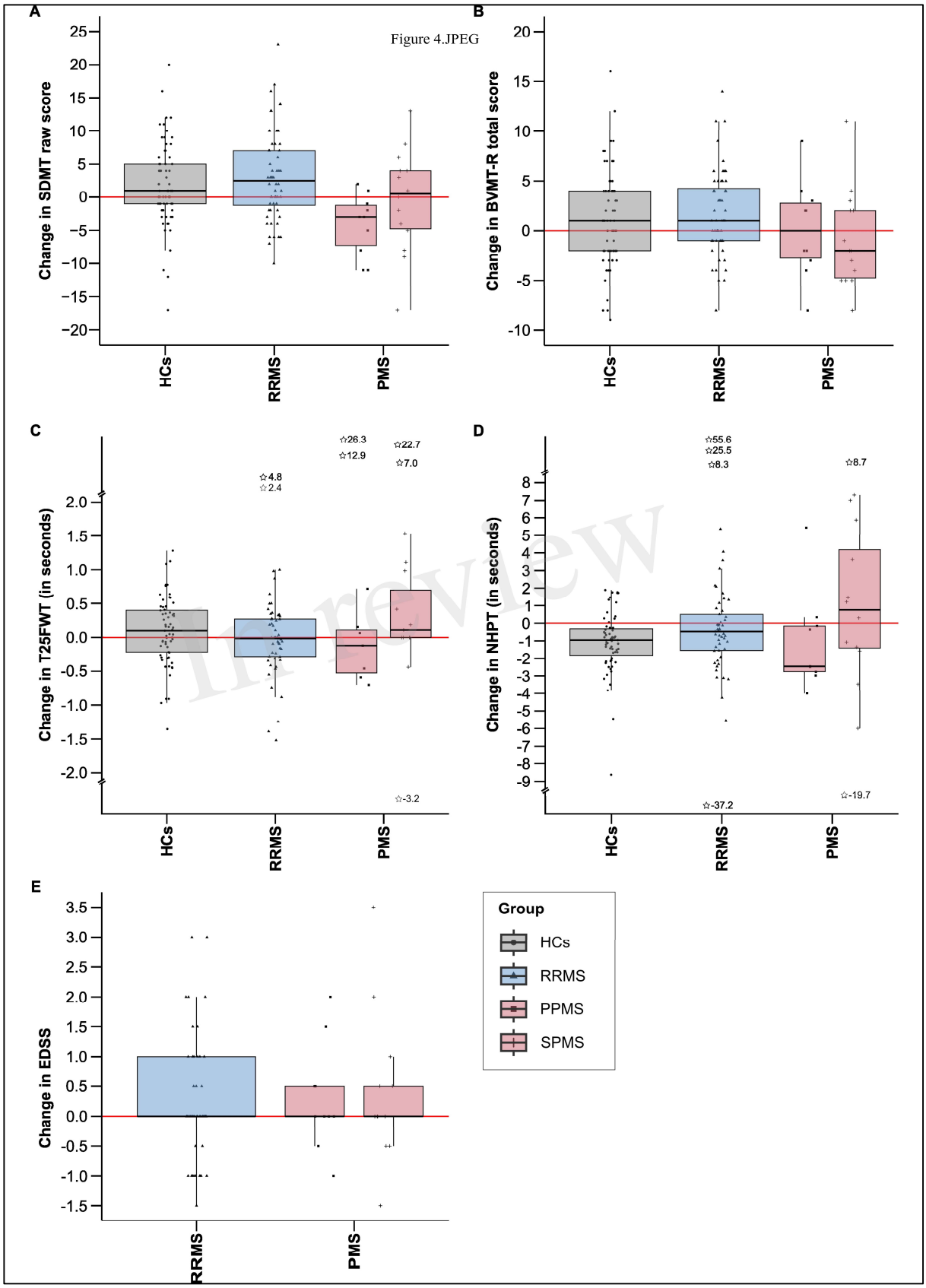
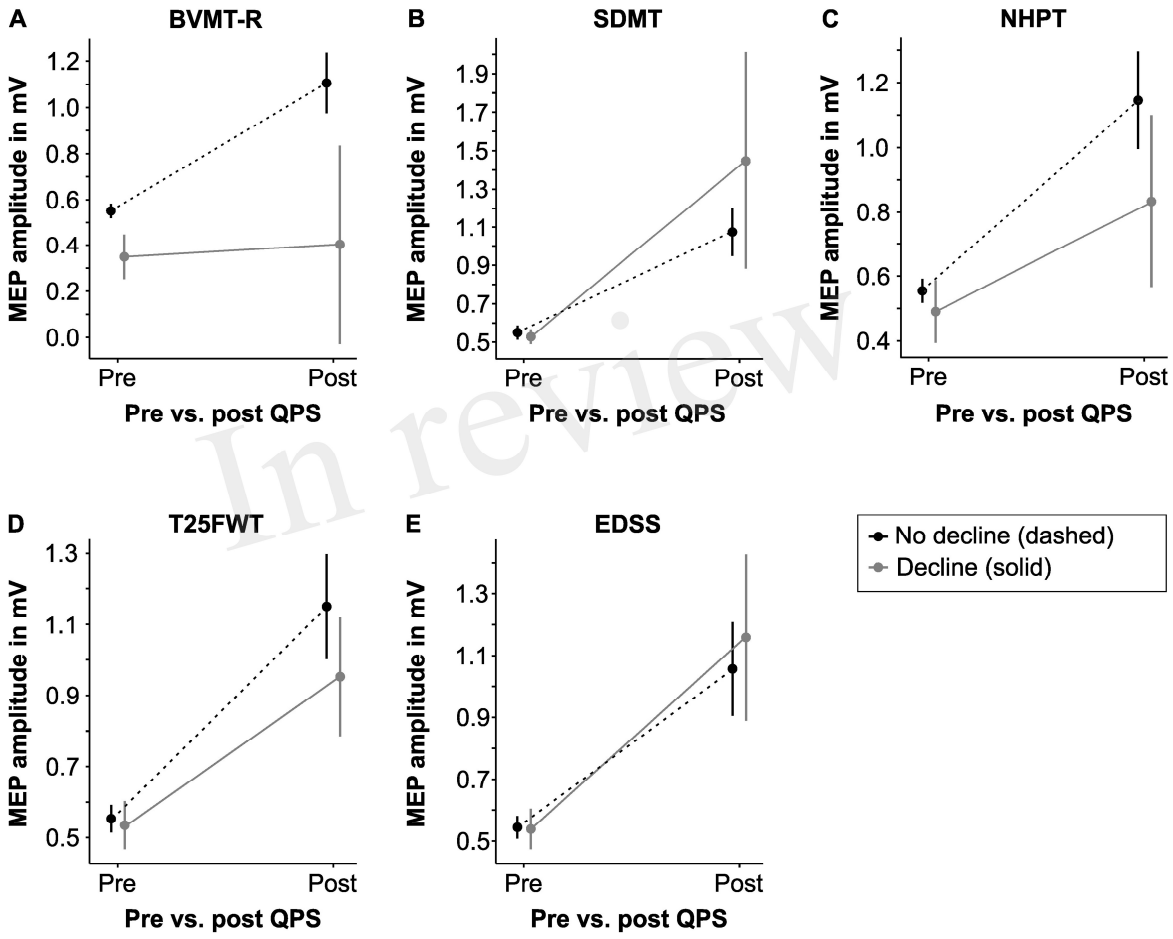

A
Figure 4.JPEG
Change in SDMT raw score
25
20
15
10
5
0
−5
−10
−15
−20
HCs
RRMS
PMS
B
Change in BVMT-R total score
20
15
10
5
0
-5
-10
HCs
RRMS
PMS
C
Change in T25FWT (in seconds)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
☆26.3
☆12.9
☆22.7
☆7.0
☆4.8
☆2.4
☆-3.2
HCs
RRMS
PMS
D
Change in NHPT (in seconds)
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
☆55.6
☆25.5
☆8.3
☆8.7
☆-37.2
☆-19.7
HCs
RRMS
PMS
E
Change in EDSS
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
RRMS
PMS
Group
HCs
RRMS
PPMS
SPMS

Figure 5.JPEG



frontiers

Supplementary Material

1 Supplementary Methods. Remote assessment

Baseline assessments were conducted in-person, while video-based neuro(psycho)logical testing was offered for follow-up evaluations. This option was provided only as a last resort for participants unable or unwilling to attend in-person assessments. Prior to remote assessments, participants received an email containing an uniform resource locator for an online video meeting. Adequate audio and video quality were required, with participants asked to minimize potential distractions, such as interruptions from family members, or phone calls.

At the beginning of each remote assessment, trained personnel conducted a structured interview to assess medical history and approximate the Expanded Disability Status Scale (EDSS) score. Patient-reported EDSS assessments and those determined by neurologists highly correlate, suggesting patient-reported evaluations as valid alternatives when physician-derived scores are unavailable (1). Modifications to one of these questionnaires (2) were made to simplify administration, including incorporation into a structured interview format and detailed assessment of functional systems.

Following the interview, cognitive tests were administered. Previous studies have shown remote administration of the Symbol Digit Modalities Test (SDMT) to yield comparable results to in-person testing, recommending it as a valid option for virtual assessment of information-processing speed (3–5).

Regarding the Brief Visuospatial Memory Test-Revised (BVMT-R), only one study has examined its virtual administration, reporting significantly higher scores in the remote setting compared to in-person testing (5). Possible factors contributing to this disparity include the use of different investigators and varied screen sizes among participants (phones vs. computer screens). In our study, investigators remained consistent across administration settings, and participants were required to have a minimum screen size of either a tablet or computer for participation.

Cognitive tests were presented via screen-sharing as PDF documents. SDMT instructions mirrored those of in-person assessments. For the BVMT-R, participants were instructed to have necessary materials prepared, including three blank sheets of DIN A4 paper, a dark pen, and an eraser. Following each trial, a screenshot of the response sheet displayed to the webcam was captured to prevent any subsequent alterations. After each trial, participants were reminded to keep their previous responses out of sight. Scoring of the geometric shapes was conducted post-assessment, adhering to the same criteria employed in in-person evaluations.

2 Supplementary Table 1. Multivariable linear mixed-effects model of MEP amplitude pre and post QPS at baseline in patients with clinically significant decline in the BVMT-R

Supplementary Material

Fixed Effects					**Random Effects**
	β-coefficient (95% CI)	*SE_b*	*t-value*	*p*	*SD*
Intercept	+0.55 (+0.51; +0.58)[a]	0.02	+34.00	**<.0001**	
Pre QPS	Reference				
Post QPS	+0.55 (+0.43; +0.68)[a]	0.07	+8.80	**<.0001**	
Stable or improved BVMT-R	Reference				
Declined BVMT-R	-0.20 (-0.31; -0.10)[a]	0.05	-3.84	**.0003**	
Fatigue	-0.05 (-0.08; -0.03)[a]	0.01	-4.41	**<.0001**	
Post QPS*Declined BVMT-R	-0.50 (-∞; -0.19)[a]	0.19	-2.67	**.005**	
Subject*Pre QPS					0.09
Subject*Post QPS					0.38
Residual					0.11

Note. Two-tailed 95% confidence intervals and *p*-values are displayed for all factors except for the primary variable of interest (Post QPS*Declined BVMT-R). For this factor, testing our hypothesis that patients with functional decline present with lower levels of QPS-induced plasticity at baseline than patients with stable performance, one-tailed 95% confidence intervals and *p*-values are reported. *p*-values <.05 are in boldface. *t*- and *p*-values are based on asymptotic Wald test. Fatigue centered at sample mean. R^2(conditional)=0.93. R^2(marginal)=0.50. Adjusted Intraclass Correlation Coefficient=0.86.
MEP= Motor evoked potential. QPS= Quadripulse stimulation. BVMT-R= Brief Visuospatial Memory Test – Revised.
[a] indicates statistical significance.

3 Supplementary Table 2. Multivariable linear mixed-effects model of MEP amplitude pre and post QPS at baseline in patients with clinically significant decline in the NHPT

Fixed Effects					**Random Effects**
	β-coefficient (95% CI)	*SE_b*	*t-value*	*p*	*SD*
Intercept	+0.55 (+0.52; +0.59)[a]	0.02	+29.41	**<.0001**	
Pre QPS	Reference				
Post QPS	+0.59 (+0.45; +0.73)[a]	0.07	+8.37	**<.0001**	
Stable or improved NHPT	Reference				
Declined NHPT	-0.06 (-0.17; +0.04)	0.05	-1.22	.23	
Post QPS*Declined NHPT	-0.25 (-∞; -0.05)	0.12	-2.06	**.02**	
Subject*Pre QPS					0.12
Subject*Post QPS					0.35
Residual					0.11

Note. Two-tailed 95% confidence intervals and *p*-values are displayed for all factors except for the primary variable of interest (Post QPS*Declined NHPT). For this factor, testing our hypothesis that patients with functional decline present with lower levels of QPS-induced plasticity at baseline than patients with stable performance, one-tailed 95% confidence intervals and *p*-values are reported. *p*-values <.05 are in boldface. *t*- and *p*-values are based on asymptotic Wald test. R^2(conditional)=0.99. R^2(marginal)=0.57. Adjusted Intraclass Correlation Coefficient=0.98.
MEP= Motor evoked potential. QPS= Quadripulse stimulation. NHPT= Nine-hole peg test.
[a] indicates statistical significance.

4 Supplementary Table 3. Multivariable linear mixed-effects model of MEP amplitude pre and post QPS at baseline in patients with clinically significant decline in the SDMT

Fixed Effects					**Random Effects**
	β-coefficient (95% CI)	*SE$_b$*	*t-value*	*p*	*SD*
Intercept	+0.55 (+0.51; +0.59)[a]	0.02	+29.50	**<.0001**	
Pre QPS	Reference				
Post QPS	+0.52 (+0.41; +0.64)[a]	0.06	+8.69	**<.0001**	
Stable or improved SDMT	Reference				
Declined SDMT	-0.02 (-0.07; +0.03)	0.03	-0.80	.43	
Latency	-0.05 (-0.08; -0.02)[a]	0.02	-3.01	**.004**	
Post QPS*Declined SDMT	+0.39 (-∞; +0.87)	0.29	+1.36	.09	
Post QPS*Latency	-0.22 (-0.33; -0.11)[a]	0.06	-3.76	**.0004**	
Subject*Pre QPS					0.04
Subject*Post QPS					0.47
Residual					0.02

Note. Two-tailed 95% confidence intervals and *p*-values are displayed for all factors except for the primary variable of interest (Post QPS*Declined SDMT). For this factor, testing our hypothesis that patients with functional decline present with lower levels of QPS-induced plasticity at baseline than patients with stable performance, one-tailed 95% confidence intervals and *p*-values are reported. *p*-values <.05 are in boldface. *t*- and *p*-values are based on asymptotic Wald test. R^2(conditional)=0.99. R^2(marginal)=0.52. Adjusted Intraclass Correlation Coefficient=0.96. Latency centered at sample mean. Correlation structure: AR(1), phi=0.86.
MEP= Motor evoked potential. QPS= Quadripulse stimulation. SDMT= Symbol Digit Modalities Test.
[a] indicates statistical significance.

5 Supplementary Table 4. Multivariable linear mixed-effects model of MEP amplitude pre and post QPS at baseline in patients with clinically significant decline in the EDSS

Fixed Effects					**Random Effects**
	β-coefficient (95% CI)	*SE$_b$*	*t-value*	*p*	*SD*
Intercept	+0.54 (+0.51; +0.58)[a]	0.02	+28.57	**<.0001**	
Pre QPS	Reference				
Post QPS	+0.51 (+0.37; +0.65)[a]	0.07	+7.14	**<.0001**	
Stable or improved EDSS	Reference				
Declined EDSS	-0.01 (-0.08; +0.07)	0.04	-0.14	.89	
Fatigue	-0.04 (-0.07; -0.01)[a]	0.02	-2.45	**.02**	
Post QPS*Declined EDSS	+0.11 (-∞; +0.35)	0.15	+0.73	.23	
Subject*Pre QPS					0.11
Subject*Post QPS					0.59
Residual					0.10

Note. Two-tailed 95% confidence intervals and *p*-values are displayed for all factors except for the primary variable of interest (Post QPS*Declined EDSS). For this factor, testing our hypothesis that patients with functional decline present with lower levels of QPS-induced plasticity at baseline than patients with stable performance, one-tailed 95% confidence intervals and *p*-values are reported. *p*-values <.05 are in boldface. *t*- and *p*-values are based on asymptotic Wald test. R^2(conditional)=0.96. R^2(marginal)=0.29. Adjusted Intraclass Correlation Coefficient=0.95. Fatigue centered at sample mean and based on the total score on the Fatigue Scale for Motor and Cognitive Functions.(6)
MEP= Motor evoked potential. QPS= Quadripulse stimulation. EDSS= Expanded Disability Status Scale.
[a] indicates statistical significance.

6 Supplementary Table 5. Multivariable linear mixed-effects model of MEP amplitude pre and post QPS at baseline in patients with clinically significant decline in the T25FWT

Fixed Effects					**Random Effects**
	β-coefficient (95% CI)	*SE_b*	*t-value*	*p*	*SD*
Intercept	+0.55 (+0.51; +0.59)[a]	0.02	+28.01	**<.0001**	
Pre QPS	Reference				
Post QPS	+0.60 (+0.46; +0.74)[a]	0.07	+8.39	**<.0001**	
Stable or improved T25FWT	Reference				
Declined T25FWT	-0.02 (-0.10; +0.06)	0.04	-0.46	.65	
Latency	-0.06 (-0.09; -0.03)[a]	0.02	-3.66	**.0005**	
Post QPS*Decline T25FWT	-0.18 (-∞; +0.02)	0.12	-1.47	.07	
Post QPS* Latency	-0.23 (-0.33; -0.13)[a]	0.05	-4.55	**<.0001**	
Subject*Pre QPS					0.09
Subject*Post QPS					0.22
Residual					0.12

Note. Two-tailed 95% confidence intervals and *p*-values are displayed for all factors except for the primary variable of interest (Post QPS*Declined T25FWT). For this factor, testing our hypothesis that patients with functional decline present with lower levels of QPS-induced plasticity at baseline than patients with stable performance, one-tailed 95% confidence intervals and *p*-values are reported. *p*-values <.05 are in boldface. *t*- and *p*-values are based on asymptotic Wald test.
R^2(conditional)=0.92. R^2(marginal)=0.78. Adjusted Intraclass Correlation Coefficient=0.63. Latency centered at sample mean. Correlation structure: AR(1), phi=0.55
MEP= Motor evoked potential. QPS= Quadripulse stimulation. T25FWT= Timed 25-foot walk test.
[a] indicates statistical significance.

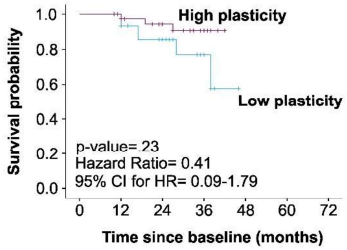
7 Supplementary Table 6. Odds ratio (including 95% confidence intervals) of clinically meaningful decline for all functional outcomes.

	Odds ratio	*Lower bound CI*	*Upper bound CI*	*p*
EDSS	1.635	0.648	4.122	.30
SDMT	1.671	0.531	5.263	.38
BVMT-R	0.008	0.000	2.329	.10
NHPT	0.185	0.018	1.938	.16
T25FWT	0.134	0.015	1.236	.08

Note. Two-tailed 95% confidence intervals and *p*-values are displayed.
EDSS= Expanded Disability Status Scale. SDMT= Symbol Digit Modalities Test. BVMT-R= Brief Visuospatial Memory Test – Revised. T25FWT = Timed 25-foot walk test. NHPT= Nine-hole peg test.

8 Supplementary Figure 1

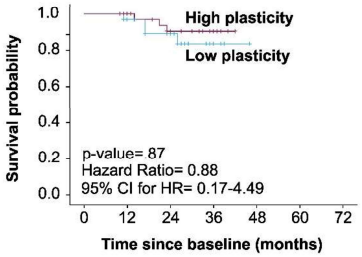
A



Patients at risk (censored)

High plasticity	41	34	26	9	0	0	0
	(0)	(6)	(13)	(29)	(38)	(38)	(38)
Low plasticity	33	25	17	4	0	0	0
	(0)	(6)	(12)	(24)	(27)	(27)	(27)

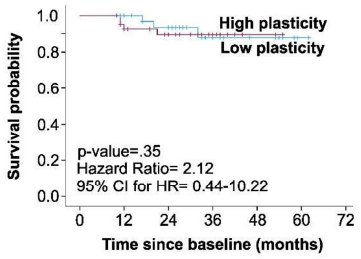
B



Patients at risk (censored)

High plasticity	41	34	25	9	0	0	0
	(0)	(7)	(13)	(29)	(38)	(38)	(38)
Low plasticity	33	26	17	4	0	0	0
	(0)	(6)	(13)	(25)	(29)	(29)	(29)

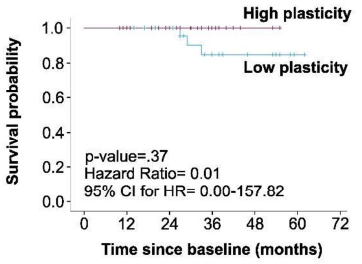
C



Patients at risk (censored)

High plasticity	42	34	26	10	2	0	0
	(0)	(5)	(12)	(28)	(36)	(38)	(38)
Low plasticity	38	32	24	13	8	1	0
	(0)	(6)	(12)	(22)	(27)	(34)	(35)

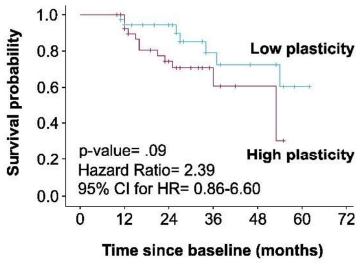
D



Patients at risk (censored)

High plasticity	42	35	27	10	2	0	0
	(0)	(7)	(15)	(32)	(40)	(42)	(42)
Low plasticity	38	32	24	14	9	1	0
	(0)	(6)	(0)	(21)	(26)	(34)	(35)

E

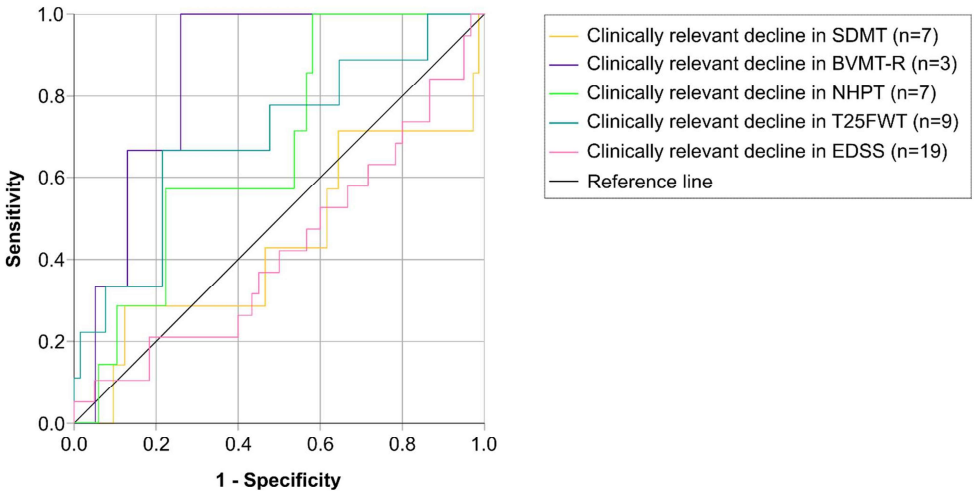


Patients at risk (censored)

High plasticity	42	32	21	6	2	0	0
	(0)	(7)	(12)	(25)	(29)	(30)	(30)
Low plasticity	37	30	22	12	7	1	0
	(0)	(5)	(11)	(20)	(24)	(29)	(30)

Note. This figure illustrates the Kaplan-Meier-Curves, Cox proportional hazard ratios, and number of remaining patients under observation for T25FWT (A), NHPT (B), SDMT (C), BVMT-R (D), and EDSS (E) from baseline to latest follow-up, stratified by high (red) vs. low (blue) plasticity at baseline. Survival probability (y-axis) represents the probability of not experiencing clinically relevant decline in the functional parameter. Age and sex were covariates in all models except for BVMT-R due to non-convergence when including these covariates.
T25FWT = Timed 25-foot walk test. NHPT= Nine-hole peg test. SDMT= Symbol Digit Modalities Test. BVMT-R= Brief Visuospatial Memory Test – Revised. EDSS= Expanded Disability Status Scale.

9 Supplementary Figure 2



Note. This figure illustrates the receiver-operating characteristic curve of the accuracy of the difference between the maximum of the six mean post MEPs and the pre MEP amplitude to differentiate between patients with and without clinically relevant decline in the SDMT, BVMT-R, NHPT, T25FW, and EDSS. Area under the curve: SDMT=.442, BVMT-R=.853, NHPT= .672, T25FW= .697, EDSS= .425.
MEP= Motor evoked potential; SDMT= Symbol Digit Modalities Test; BVMT-R= Brief Visuospatial Memory Test – Revised; NHPT= Nine-hole peg test; T25FWT= Timed 25-foot walk test; EDSS= Expanded Disability Status Scale

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Appendix C: List of further publications

In addition to the studies included in this thesis, I published the following research articles:

Balloff, C., Bandlow, C., Bernhard., M., Brandenburger, T., Conrads, P., Elben, S., Feldt, T., Hartmann, C.J., Heinen, E., Ingwersen, J., Jansen, C., Jensen, B.E.O., Kindgen-Milles, D., Luedde, T., Penner, I.K., Slink, I., Stramm, K., Telke, A.K., … Groiss, S.J.,[9] & Albrecht, P.[9] (2023) Prevalence and prognostic value of neurological affections in hospitalized patients with moderate to severe COVID-19 based on objective assessments. *Scientific Reports, 13,* 19619. https://doi.org/10.1038/s41598-023-46124-w

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Appendix D: Affidavit

Eidesstattliche Erklärung gemäß § 5 der Promotionsordnung vom 15.06.2018 der Mathematisch-Naturwissenschaftlichen Fakultät der Heinrich-Heine-Universität Düsseldorf:

Ich versichere an Eides Statt, dass die Dissertation von mir selbstständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherheit guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist.

Ich habe die Arbeit weder in der vorliegenden noch in ähnlicher Form oder auszugsweise im Rahmen einer anderen Prüfung vorgelegt. Ich versichere, dass ich bisher keine erfolglosen Promotionsversuche unternommen habe.

Düsseldorf, den 10.05.2024