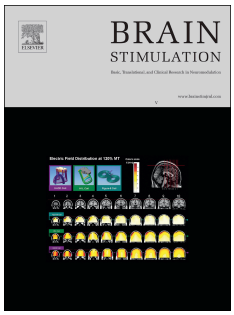

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The degree of cortical plasticity correlates with cognitive performance in patients with Multiple Sclerosis

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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.

Keywords: cortical plasticity; cognition; Multiple Sclerosis; repetitive transcranial magnetic stimulation; quadripulse stimulation

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 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.

Materials and methods

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.

Experimental design

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

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.

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 ten minutes during a follow up of 60 minutes (Figure A.1, *Supplement*). The degree of MEP amplitude changes induced by QPS served as measure of cortical plasticity.

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 BiStim² (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 5cm lateral and 1cm 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 ≥ 50μV 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 ≥ 100μV 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]

Motor evoked potential recordings

MEPs were recorded by surface EMG using Ag-AgCl-electrodes (20x15 mm, Ambu, Ballerup, Denmark) in a belly-tendon montage. The signal was amplified (Digitimer D360, Digitimer 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.

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 200², 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 5ms, 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.

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]

Data availability

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

Results

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 (Figure 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.

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 Figure 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 (β=1.20, p=.03) and SDMT (β=2.83, p=.006) (Table 3).

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

Figure 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 (β= 0.63, p<.001) and age (β= -0.03, p=.03) (Table 4). Prior to stimulation, MEP amplitudes were equal in both groups (β=-0.01, p=.80) since they were experimentally adjusted to be ~0.5mV 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 (Figure 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.

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*.

Figure 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 A.2, *Supplement*).

As described above, group differences were tested for statistical significance using linear mixed-effects models. The model including QPS, group, fatigue, and QPS^{x}group was superior compared to all other models and revealed significant effects of QPS intervention (β=0.69, p<.001), fatigue (β=–0.04, p=.03), and QPS^{x}group (β=–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.

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 Figure 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 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 (Figure 4).

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 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.

Figure captions

Figure 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

Figure 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

Figure 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

Figure 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online.

Supplementary figure captions

Figure A.1. Illustration of the QPS protocol

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

Figure A.2. QPS-induced plasticity in RRMS patients with impairment in the SDMT (a) and BVMT (b) compared to patients without impairment in these tests

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

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Tables

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 0.5mV, 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 0.5mV= 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: 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]

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				
Depression	-0.08	.54	-0.16	.26
Anxiety	0.19	.14	-0.23	.09
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.

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 $R^2_{BVMT\text{-}R}$=.38 (*p*<.001). Adjusted R^2_{SDMT}=.37 (*p*<.001).
[a] male=0, female=1

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.

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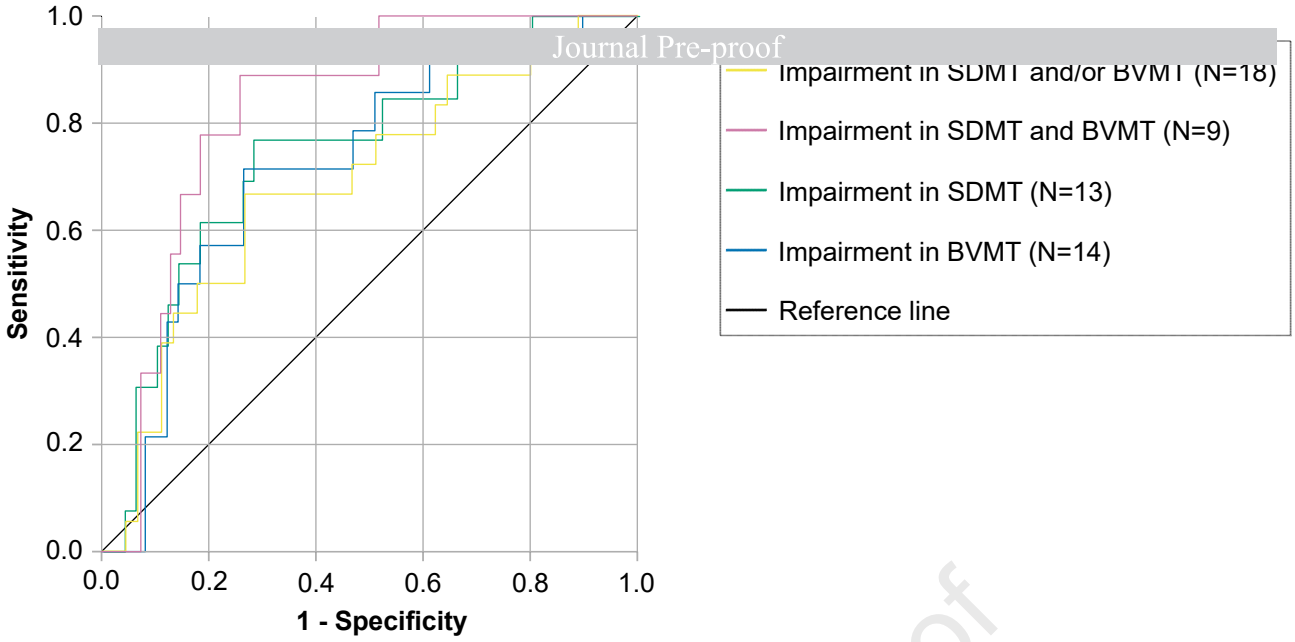

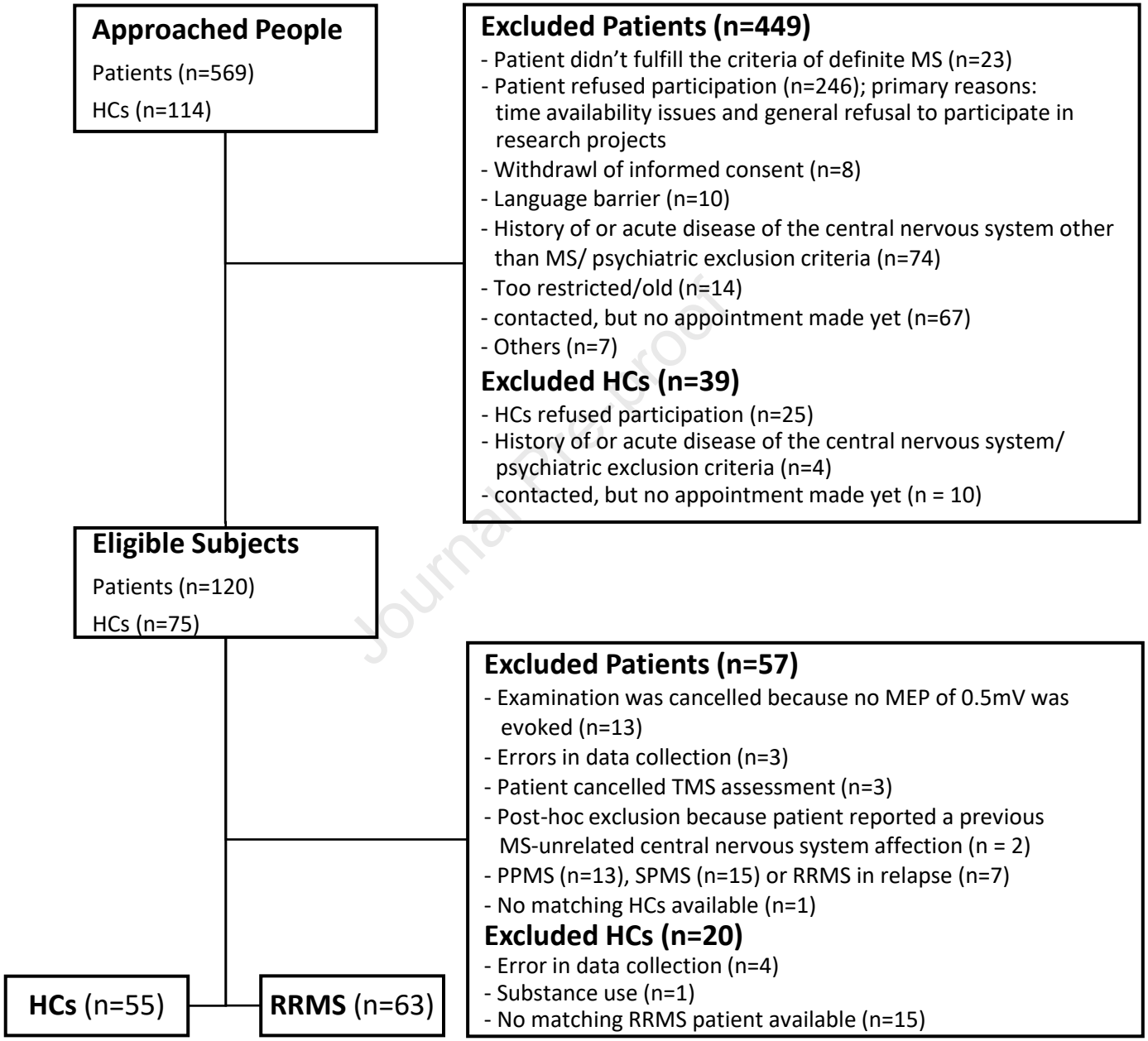

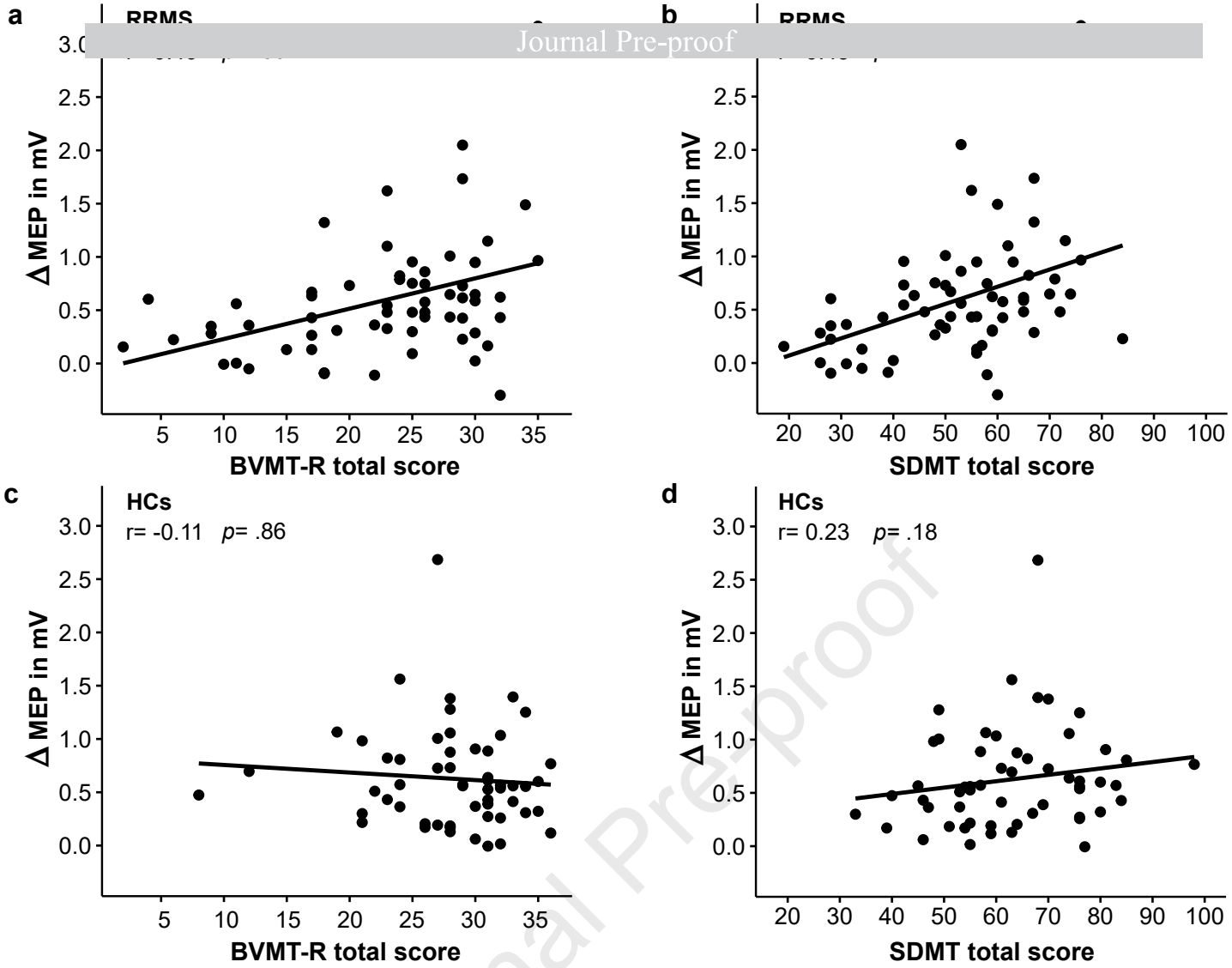

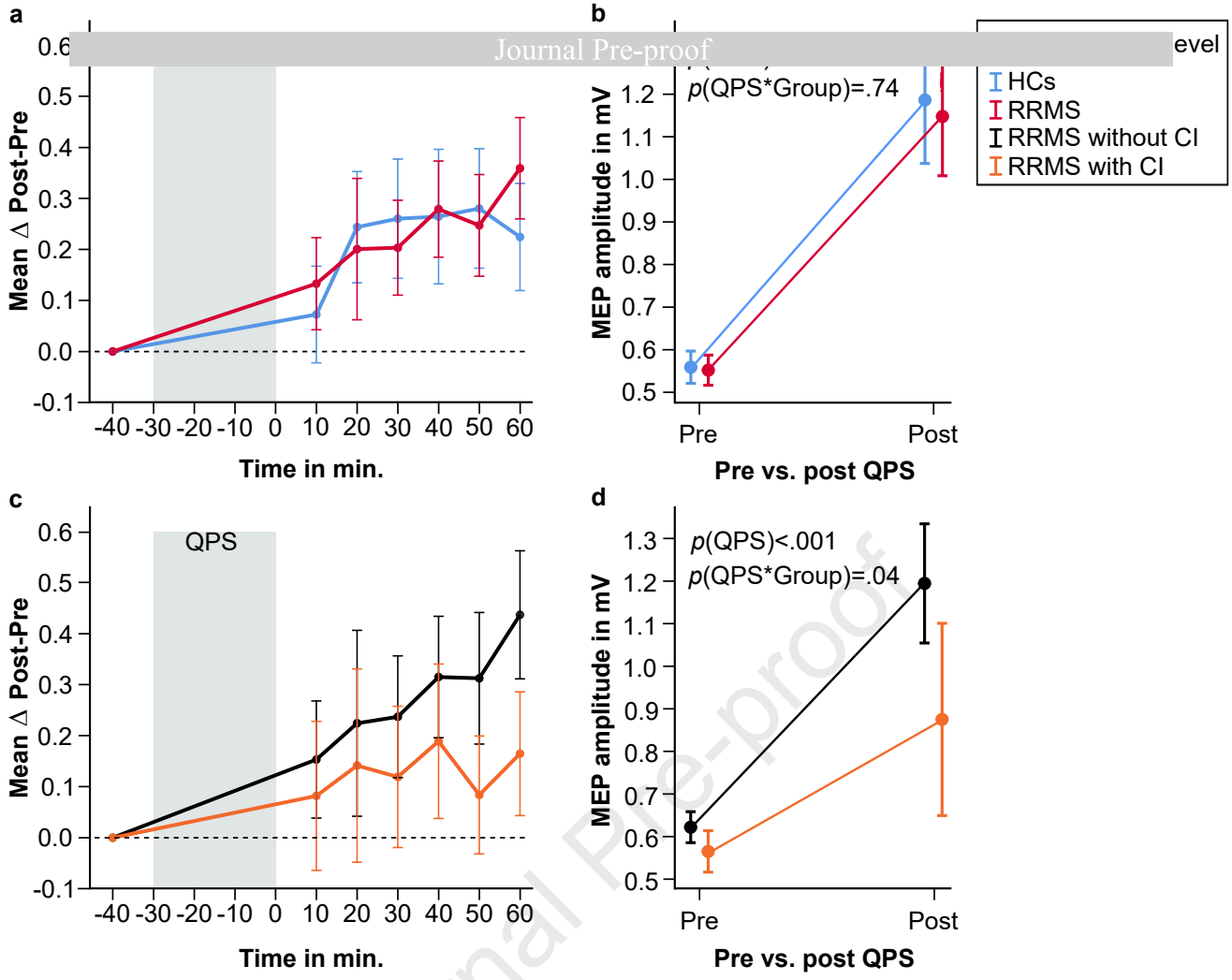

Highlights

- QPS-induced plasticity correlates with cognitive performance in RRMS patients
- Cognitively impaired RRMS patients are characterized by reduced plasticity
- QPS may be apt to investigate the prognostic value of cortical plasticity in MS

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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