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ORIGINAL ARTICLE

# Preventive exercise and physical rehabilitation promote long-term potentiation-like plasticity expression in patients with multiple sclerosis

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## Abstract

**Background and purpose:** Loss of long-term potentiation (LTP) expression has been associated with a worse disease course in relapsing–remitting multiple sclerosis (RR-MS) and represents a pathophysiological hallmark of progressive multiple sclerosis (PMS). Exercise and physical rehabilitation are the most prominent therapeutic approaches to promote synaptic plasticity. We aimed to explore whether physical exercise is able to improve the expression of LTP-like plasticity in patients with multiple sclerosis (MS).

**Methods:** In 46 newly diagnosed RR-MS patients, we explored the impact of preventive exercise on LTP-like plasticity as assessed by intermittent theta-burst stimulation. Patients were divided into sedentary or active, based on physical activity performed during the 6 months prior to diagnosis. Furthermore, in 18 patients with PMS, we evaluated the impact of an 8-week inpatient neurorehabilitation program on clinical scores and LTP-like plasticity explored using paired associative stimulation (PAS). Synaptic plasticity expression was compared in patients and healthy subjects.

**Results:** Reduced LTP expression was found in RR-MS patients compared with controls. Exercising RR-MS patients showed a greater amount of LTP expression compared with sedentary patients. In PMS patients, LTP expression was reduced compared with controls and increased after 8 weeks of rehabilitation. In this group of patients, LTP magnitude at baseline predicted the improvement in hand dexterity.

**Conclusions:** Both preventive exercise and physical rehabilitation may enhance the expression of LTP-like synaptic plasticity in MS, with potential beneficial effects on disability accumulation.

KEYWORDS
exercise, multiple sclerosis (MS), physical rehabilitation, synaptic plasticity, transcranial magnetic stimulation (TMS)

## INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by demyelination, axonal loss and neurodegeneration [1, 2]. Relapsing–remitting MS (RR-MS) is characterized by the onset of acute neurological deficits followed by full or incomplete recovery [3]. The disease, after a fluctuating period of relapses and remissions, may evolve to a progressive accumulation of disability without obvious relapses (secondary progressive MS), or it may show a progressive course from onset (primary progressive MS [PMS]).

Synaptic plasticity, in particular long-term potentiation (LTP), is a key physiological mechanism involved in the clinical compensation of brain damage [4, 5]. Loss of LTP expression in RR-MS patients has been associated with reduced ability to compensate for newly formed inflammatory brain lesions and worse disease course [6, 7]. Notably, loss of synaptic plasticity may characterize RR-MS patients at risk of progression [8], and represents a pathophysiological hallmark of PMS [9, 10].

Restoring synaptic plasticity may improve MS course by promoting LTP induction and LTP-dependent functional compensation of deficits, thereby slowing down the neurodegeneration process. Exercise and physical rehabilitation are the most prominent therapeutic approaches to promote synaptic plasticity and improve clinical recovery in patients with neurological deficits. Physical exercise has been suggested as an effective treatment strategy for people with MS [11–13], indeed studies in animal models of MS have demonstrated that different exercise paradigms, such as environmental enrichment-mimicking lifestyle interventions, voluntary and forced exercise, both preventive and therapeutic, can restore LTP expression and improve functional outcomes [14, 15].

In a group of newly diagnosed RR-MS patients, we examined whether preventive exercise, performed in the 6 months prior to MS diagnosis, could be associated with greater expression of LTP-like plasticity, explored using transcranial magnetic stimulation (TMS). In a group of patients with PMS, we also assessed whether an 8-week motor rehabilitation program could enhance LTP-like plasticity.

## METHODS

### Patients

Forty-six newly diagnosed RR-MS patients and 18 PMS patients, admitted to the Neurology Clinic of IRCCS Neuromed (Pozzilli, Italy) between 2018 and 2020, participated in the study. The local ethics committee approved the study (CE number 06/17), and all patients gave their written informed consent. All procedures were performed according to approved guidelines. The following demographic and clinical characteristics were recorded: age, sex, disease duration, clinical disability evaluated using the Expanded Disability Status Scale (EDSS), and presence of oligoclonal bands in the cerebrospinal fluid (CSF) [16]. In patients with PMS, the nine-hole pegboard test (9-HPT), a timed 25-foot walk test (T25FW), and the Fatigue Severity Scale (FSS) [17] were used before and after rehabilitation. All clinical evaluations were performed by certified neurologists experienced in MS, who were blinded to physical activity level in the RR-MS group and to TMS results in both the RR-MS and PMS groups.

The RR-MS patients were clinically stable at the time of evaluation and had no sensory-motor deficits in the dominant upper and lower limbs. No patients were treated with corticosteroids or disease-modifying therapies in the 6 months prior to clinical and neurophysiological evaluations.

Magnetic resonance imaging (1.5 or 3.0 Tesla) was performed in all MS patients, including dual-echo proton density, fluid-attenuated inversion recovery, T1-weighted spin-echo, T2-weighted fast spin-echo, and contrast-enhanced T1-weighted spin-echo after intravenous gadolinium infusion.

### Preventive exercise and physical rehabilitation

The RR-MS patients were interviewed about the physical activity they had performed in the 6 months prior to diagnosis. They were divided into two groups (sedentary and exercise) based on the type of activity and the number of hours performed [18]. The exercise group comprised patients who performed at least 150 min/week of repetitive physical activity (e.g., jogging, swimming).

The PMS patients received a therapeutic exercise program at the Neurorehabilitation Department of IRCCS Neuromed. Physical therapy was performed for 6 days/week for 8 weeks and consisted of 3-h daily treatment. The rehabilitation program, planned by a physical medicine and rehabilitation physician according to the patient's disability status, included passive and active exercises specifically aimed at restoring or maintaining muscle flexibility, range of motion, balance, coordination of movements, postural passages, and walking ability. Exercises included: repetition of different movements (e.g., toes and heels, 90° flexed hips and knees) for walking and stair climbing, repetition of crossed-movement patterns for coordination, standing postural reactions with eyes open and closed and oscillatory boards for balance, strengthening of lower limb muscles, and low-intensity and long-duration static stretching of iliopsoas, rectus femoris, hamstrings, triceps surae, and lumbar spinal muscles for muscle flexibility and range of motion. Rehabilitation treatment was tailored to achieve in each patient the maximum level of intensity without inducing fatigue. Each exercise consisted of 1–3 sets of 8–15 repetitions, and the intensity was between 11 and 13 on the 20-point Perceived Exertion Rating Scale [19], without exceeding 15 points. To increase the patient's tolerance, compensative pauses were included.

### Transcranial magnetic stimulation

In patients with RR-MS, LTP-like synaptic plasticity was studied using the intermittent theta-burst stimulation (iTBS) protocol [20].

Motor evoked potentials (MEPs) were elicited using a figure-of-eight coil, with an external loop diameter of 70 mm, connected to a Magstim 200$^2$ magnetic stimulator (The Magstim Company). The coil was positioned in the optimal position (hot spot) for eliciting MEPs in the right first dorsal interosseus (FDI) muscle. iTBS was applied to the right FDI motor hot spot using a Magstim Rapid$^2$ stimulator, with a stimulation intensity of 80% of the active motor threshold (AMT) [20]. The AMT was calculated as the minimum stimulus intensity for evoking MEPs of approximately 200 μV in 50% of 10 consecutive trials from slightly contracted FDI muscle. As the after-effects of TBS could be affected by the prior functional state of cortical motor neurons [21–24], to prevent tonic muscle contraction during AMT assessment from influencing the effects of subsequent iTBS, we determined AMT at least 15 min before delivering iTBS. Twenty MEPs were recorded from the relaxed FDI muscle prior to iTBS, with intensity set to elicit stable MEPs of 0.5–1 mV amplitude. Using the same stimulation intensity, 20 MEPs were collected 5 (post 5), 15 (post 15), and 30 (post 30) min after iTBS. At each time point, MEPs were averaged and normalized to the mean baseline amplitude. The neurophysiological results obtained in RR-MS patients were compared with those obtained in a group of 15 age- and sex-matched exercising healthy subjects.

In patients with PMS, LTP-like synaptic plasticity was evaluated using the paired associative stimulation (PAS) protocol [25]. Median nerve electric stimulation was coupled with single TMS pulses delivered over the hot spot of the abductor pollicis brevis (APB) muscle. The median nerve was stimulated at the wrist through surface electrodes with a constant current stimulator (model DS7A, Digitimer Ltd; cathode proximal, duration 0.2 ms, intensity 300% of the perceptual threshold). The interstimulus interval between the electric stimulation and the TMS pulse was 25 ms. Two hundred pairs of electric and magnetic stimuli were repetitively delivered at a rate of 0.25 Hz. TMS intensity was set to evoke MEPs of approximately 0.5–1 mV peak-to-peak amplitude in the APB muscle at baseline. The same intensity was used to elicit MEPs 5, 15, and 30 min after PAS (post 5, post 15, and post 30). MEP amplitudes were averaged and normalized to the mean baseline amplitude at each time interval. Results were compared with those obtained in a group of 14 age- and sex-matched sedentary healthy individuals.

The MEPs were recorded with surface electrodes placed on the target muscles, sampled at 5 KHz with a CED 1401 A/D laboratory interface (Cambridge Electronic Design), and amplified and filtered (bandpass 20 Hz to 2 kHz) with a Digitimer D360 (Digitimer Ltd), then recorded by a computer with Signal software (Cambridge Electronic Design).

### Statistical analysis

The Kolmogorov–Smirnov test was applied to test the normality distribution of the continuous variables.

Continuous variables are presented as mean (standard deviation [SD]) or, if necessary, as median (25–75th percentiles). Categorical data are presented as frequency (%). Differences in continuous variables were evaluated by *t*-test or, when necessary, by nonparametric Mann–Whitney test. Associations between two categorical variables were tested using the chi-squared test.

To evaluate iTBS-induced changes in MEPs size, repeated-measures analysis of variance (ANOVA) was applied assuming time (baseline, post 5, post 15, post 30) as a within-subject factor and group (controls, RR-MS) as a between-subject factor. To evaluate the effect of preventive exercise, repeated-measures ANOVA was applied using time (baseline, post 5, post 15, post 30) as a within-subject factor and group (Exercising RR-MS, Sedentary RR-MS) as between-subject factor. To evaluate PAS-induced changes in MEPs size, repeated-measures ANOVA was applied assuming time (baseline, post 5, post 15, post 30) as a within-subject factor and group (controls, PMS) as a between-subject factor. To evaluate the effect of 8 weeks of rehabilitation on LTP-like plasticity, repeated-measures ANOVA was applied using time (baseline, post 5, post 15, post 30) and condition (baseline, post-rehabilitation) as a within-subject factor. For each repeated-measures ANOVA, Mauchly's test of sphericity was applied and, if the assumption was not met, Greenhouse–Geisser (G–G) correction was used.

A *t*-test for paired data was applied to test possible changes in EDSS, 9-HPT, T25FW and FSS scores in patients with PMS after rehabilitation. Non-parametric Spearman's correlation was calculated to evaluate associations between LTP-like plasticity and changes in EDSS, 9-HPT, T25FW and FSS scores after rehabilitation. For multiple comparisons, Benjamini–Hochberg (B–H) correction was applied to the *p* value. For all analyses, $p$ values $< 0.05$ were taken to indicate statistical significance. All analyses were performed with IBM SPSS statistics for Windows, Version 20.0.

## RESULTS

### Preventive exercise and synaptic plasticity in patients with RR-MS

The characteristics of RR-MS patients and control subjects are shown in Table 1.

No significant differences were found in age and sex distribution between MS patients and controls. In addition, demographic and clinical characteristics did not differ between exercising and sedentary RR-MS patients.

We first analyzed the response to iTBS in RR-MS and control subjects, adjusting for age effect. Repeated-measures ANOVA showed a significant effect of time (time effect: $F_{G-G}$ [2.18, 126.5] = 40.748; $p < 0.001$), as MEP amplitude significantly increased in the two groups after iTBS. In addition, a significant interaction time × group (time × group effect: $F_{G-G}$ [2.18, 126.5] = 11.951; $p < 0.001$) showed that the effect of iTBS was significantly higher in the control group compared with the RR-MS patients (Figure 1a).

To explore the impact of preventive exercise, we then compared the response to iTBS in exercising and sedentary RR-MS patients.

Repeated-measures ANOVA showed a significant time effect (time effect: $F_{G-G}$ [2.269, 97.55] = 20.417; $p < 0.001$) and time × group interaction (time × group effect: $F_{G-G}$ [2.269, 97.55] = 11.56; $p < 0.001$), indicating that the effect of iTBS was significantly greater in exercising RR-MS patients compared with sedentary RR-MS patients (Figure 1b).

## Physical rehabilitation and synaptic plasticity in patients with PMS

The characteristics of PMS patients and control subjects are shown in Table 2. No significant differences were found in demographic characteristics between MS patients and controls.

Evaluation of LTP-like synaptic plasticity at baseline evidenced a significantly reduced response to PAS in PMS patients compared with healthy subjects (Figure 2a). Accordingly, repeated-measures ANOVA showed a significant time effect (time effect: $F_{G-G}$ [2.021, 58.598] = 14.790; $p < 0.001$) and time × group interaction (time × group effect: $F_{G-G}$ [2.021, 58.598] = 18.895; $p < 0.001$), adjusting for age effect.

To assess the effect of motor rehabilitation on LTP-like plasticity expression we compared the response to PAS in PMS patients before and after 8 weeks of rehabilitation. A significantly increased response to PAS has been evidenced after rehabilitation (Figure 2b). Accordingly, repeated-measures ANOVA showed a significant effect of time (time effect: $F_{G-G}$ [1.433, 24.362] = 22.683; $p < 0.001$) and a significant effect of condition (pre- vs. post-rehabilitation) (condition effect: $F$ [1, 17] = 39.461; $p < 0.001$). Finally, a significant interaction time × condition has been evidenced (time × condition: $F_{G-G}$ [2.39, 40.69] = 7.995; $p = 0.001$).

In PMS patients, no significant differences were found in 9-HPT and T25FW scores, comparing baseline and post-rehabilitation assessment (all $p > 0.1$). A significant reduction was observed in EDSS score ($p_{B-H\ adjusted} = 0.016$) and FSS score ($p_{B-H\ adjusted} = 0.022$; Figure 3a–d). Finally, a significant correlation was found between LTP at baseline and the improvement in the 9-HPT score of the dominant hand (Spearman's $r = -0.657$, $p_{B-H\ adjusted} = 0.012$, $N = 18$; Figure 3e).

No significant associations emerged between the LTP-like effect at baseline and changes of other clinical scores (T25FW: Spearman's $r = -0.014$, $p = 0.96$; FSS: Spearman's $r = -0.020$, $p = 0.94$). In addition, no significant correlations were found between the enhancement of LTP-like plasticity induced by rehabilitation and the degree of clinical improvement (Spearman's $r = -0.15$, $p = 0.55$).

# DISCUSSION

Synaptic plasticity is involved in remodeling neuronal connectivity following brain damage. Accordingly, TMS studies have shown that efficiency of LTP-like plasticity mechanisms, defined as plasticity reserve, was correlated with clinical recovery after stroke or MS relapse [5, 6] and may represent an important factor influencing the disease course of MS [26].

Exercise promotes synaptic plasticity expression [27], and may represent a useful approach to enhancing plasticity reserve in MS. It has been shown that induction of LTP-like plasticity using PAS was blocked by prior motor practice, suggesting that practice-dependent LTP mechanisms likely mediate the beneficial effects of physical exercise [28]. Moreover, enhanced LTP-like plasticity was evidenced in physically active healthy individuals compared with sedentary subjects [27, 29]. In addition, a previous study in patients with major depression reported that 3 weeks of physical activity significantly increased response to PAS [30], without affecting excitatory and inhibitory synaptic transmission, thereby suggesting a direct effect on LTP-like plasticity expression not mediated by increased neuronal recruitment or reduced GABAergic activity [30].

We explored whether preventive exercise was associated with increased LTP-like plasticity reserve in a group of newly diagnosed RR-MS patients. LTP-like plasticity induced by iTBS was reduced in RR-MS patients compared with a group of age- and sex-matched healthy controls. In addition, in RR-MS patients, physical exercise was associated with enhanced response to iTBS, as exercising RR-MS patients showed an increased amount of iTBS-induced LTP-like plasticity compared with sedentary patients. Several TMS studies have explored synaptic plasticity expression in RR-MS patients

**TABLE 1** Clinical characteristics of relapsing–remitting multiple sclerosis patients and controls.

| | Controls | RR-MS | *p* | RR-MS exercise group | RR-MS sedentary group | *p* |
|---|---|---|---|---|---|---|
| *N* | 15 | 46 | | 20 | 26 | |
| Age, years, median (IQR) | 29.45 (24.78–38.22) | 33.96 (26.31–44.6) | 0.331[a] | 32.9 (24.54–43) | 34.32 (28.42–45.40) | 0.425[a] |
| Sex: female, *n* (%) | 10 (66.7) | 32 (69.6) | 0.833[b] | 12/20 (60) | 20 (76.9) | 0.216[b] |
| Disease duration, months, median (IQR) | - | 4.8 (2.3–13.4) | - | 6.13 (1.63–12.03) | 4.31 (3.06–20.6) | 0.525[a] |
| EDSS score, median (IQR) | - | 1 (1–2) | - | 1 (1–2) | 1 (1–2) | 0.933[a] |
| OCB, yes, *n* (%) | - | 35 (76.1) | - | 14 (70) | 21 (80.8) | 0.396[b] |

Abbreviations: EDSS, Expanded Disability Status Scale; OCB, oligoclonal band; RR-MS, relapsing–remitting multiple sclerosis.

[a]Mann–Whitney.

[b]Chi-squared.

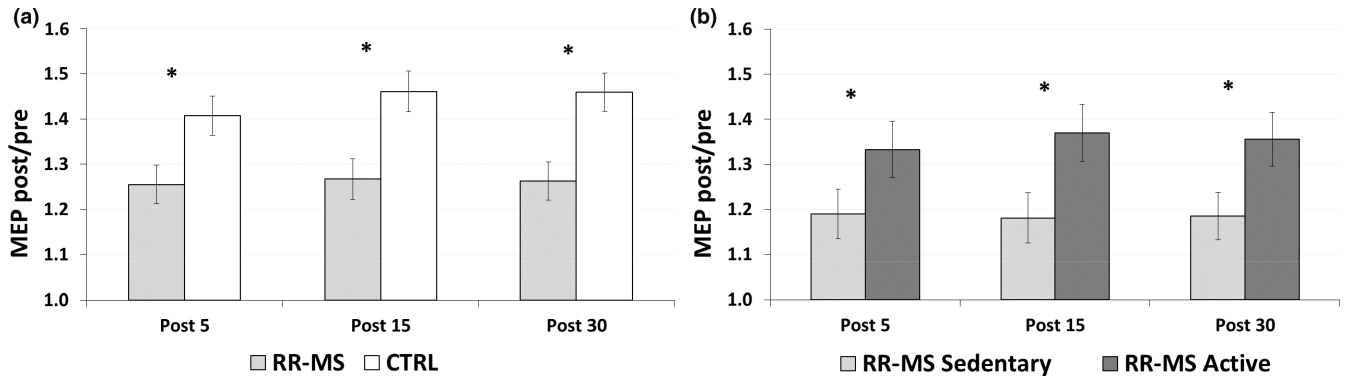


**FIGURE 1** Preventive exercise and long-term potentiation (LTP)-like plasticity in relapsing–remitting multiple sclerosis (RR-MS) patients. (a) Response to intermittent theta burst stimulation in RR-MS patients and controls. A significant difference was found between RR-MS patients and controls at each time point (post 5, $p = 0.001$; post 15, $p < 0.001$; post 30, $p < 0.001$). (b) LTP-like plasticity in sedentary and exercising RR-MS patients. A significant difference was found between sedentary and exercising RR-MS patients (post 5, $p = 0.002$; post 15, $p < 0.001$; post 30, $p < 0.001$). CTRL, controls; MEP, motor evoked potential.

**TABLE 2** Clinical characteristics of progressive multiple sclerosis patients and controls.

| | Controls | PMS | *p* |
|---|---|---|---|
| *N* | 14 | 18 | |
| Age, years, median (IQR) | 36.25 (28.2–43.12) | 40.95 (33.75–49.09) | 0.135[a] |
| Sex: female, *n* (%) | 9 (64.3) | 11 (61.1) | 0.854[b] |
| Disease duration, months, median (IQR) | - | 43.3 (29.7–62.2) | - |
| EDSS score, median (IQR) | - | 3 (2–4) | - |
| OCB, yes, *n* (%) | - | 15 (83.3) | - |

Abbreviations: EDSS, Expanded Disability Status Scale; OCB, oligoclonal band; PMS, progressive multiple sclerosis.

[a]Mann–Whitney.

[b]Chi-squared.

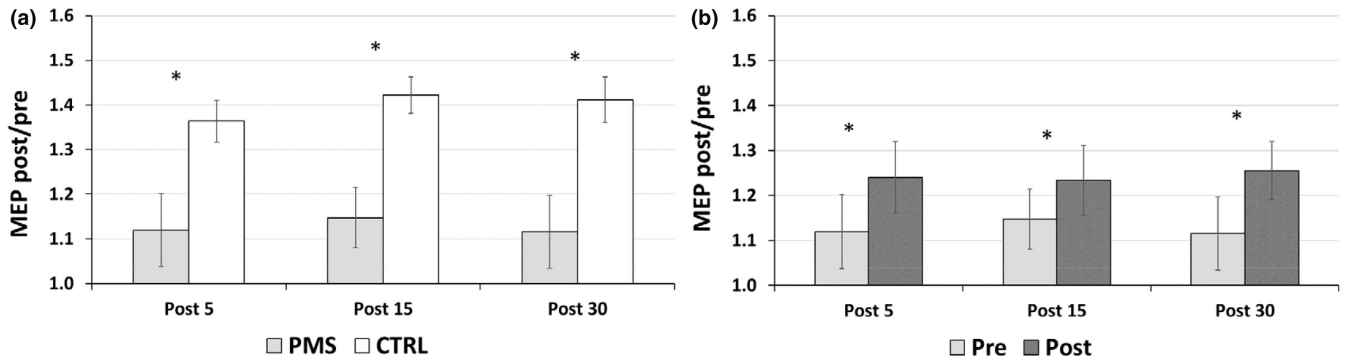


**FIGURE 2** Physical rehabilitation and long-term potentiation (LTP)-like plasticity in progressive multiple sclerosis (PMS) patients. (a) Response to paired associative stimulation (PAS) in PMS patients and controls. A significant difference was found between PMS patients and controls at each time point (post 5, $p < 0.001$; post 15, $p < 0.001$; post 30, $p < 0.001$). (b) LTP-like plasticity in PMS patients before and after rehabilitation. In PMS patients a significant difference was found at each time point comparing the response to PAS before and after rehabilitation (post 5, $p < 0.001$; post 15, $p = 0.008$; post 30, $p < 0.001$). CTRL, controls; MEP, motor evoked potential.

using different experimental protocols. Although some studies have reported preserved synaptic plasticity [31, 32], a reduced amount of LTP-like plasticity in response to iTBS, PAS, and motor practice has been consistently evidenced in RR-MS patients [7, 26, 33] and has been associated with impaired compensation of ongoing brain damage and disability accumulation. Neuroinflammation may play a prominent role in disrupting synaptic plasticity expression in MS [1, 34]. Accordingly, LTP-like plasticity is altered during acute relapses and in RR-MS patients with higher CSF levels of proinflammatory molecules [7, 33]. Hence, our results suggest that preventive exercise

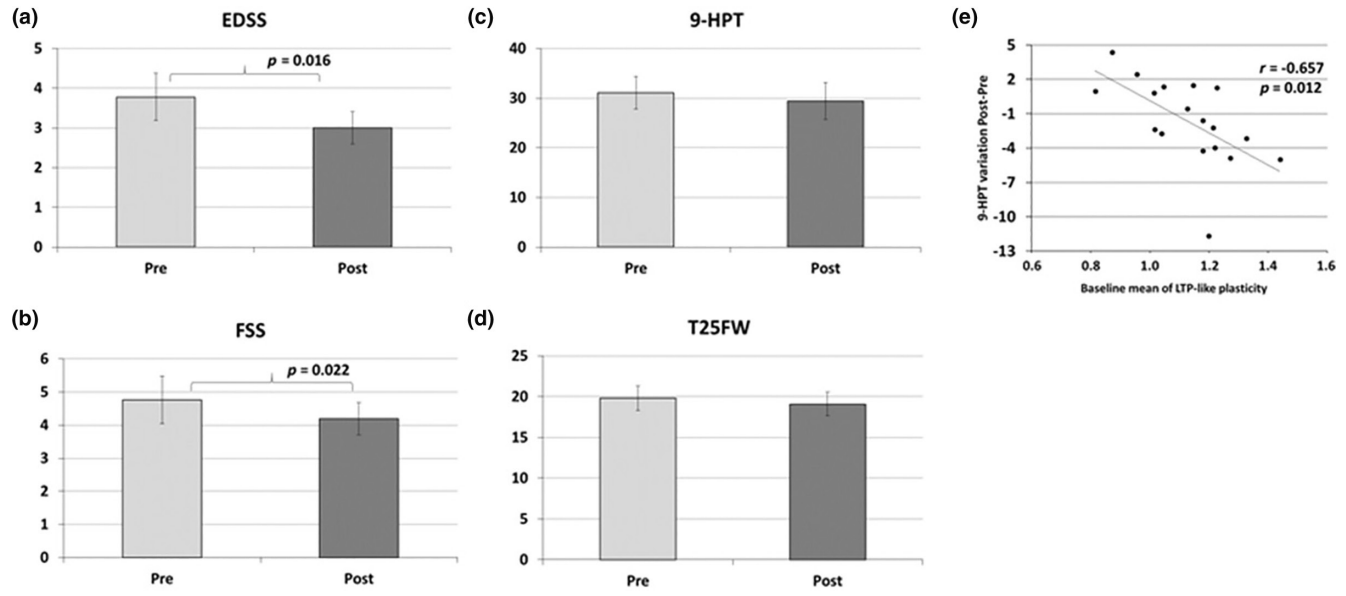


**FIGURE 3** Physical rehabilitation and clinical evaluation in progressive multiple sclerosis (PMS) patients. Changes in clinical scores after rehabilitation in PMS patients. (a–d) A significant difference was found in Expanded Disability Status Scale (EDSS) and Fatigue Severity Scale (FSS) scores before and after rehabilitation, whereas no significant differences emerged in nine-hole pegboard test (9-HPT) and timed 25-foot walk test (T25FW) scores. (e) A significant correlation was found between long-term potentiation (LTP) at baseline and the improvement in the 9-HPT score of the dominant hand after rehabilitation.

may promote LTP expression and possibly exert a protective role against the alterations of synaptic plasticity observed in RR-MS.

Exercise modulates various signaling pathways critically involved in synaptic plasticity induction. In animal models of brain damage, exercise and environmental enrichment promoted synaptic remodeling, enhanced connectivity and LTP in surviving neurons, and were associated with an increased number of dendrites and dendritic spines [35]. Notably, in experimental models of MS, exercise has been associated with both reduced dendritic spine loss and improved disease course [36].

Exercise may exert immunomodulatory effects in MS [37, 38]. Preventive exercise in experimental autoimmune encephalomyelitis (EAE) has been associated with increased expression of regulatory T cells, reduced activation of peripheral and brain resident immune cells, and decreased production of proinflammatory cytokines [39]. The beneficial effects of exercise on synaptic plasticity expression in MS may indeed result from limiting inflammation-induced synaptic changes. In line with this, we have recently reported that preventive exercise is associated with reduced CSF expression of the proinflammatory molecules interleukin (IL)-2 and IL-6 in RR-MS patients at the time of diagnosis [40]. Notably, these two molecules have been previously involved in LTP disruption in MS and identified as biomarkers related to poor compensation and disability accumulation [7].

Physical activity has also been associated with changes in brain metabolism and oxygenation, and modifications in the expression of various neurotransmitters and neurotrophic factors [41, 42]. Modulation of the endocannabinoid system plays an important role in mediating the effects of exercise on LTP. In fact, exercise is associated with increased endocannabinoid levels [43], and altered activity of the cannabinoid receptor type 1 in RR-MS patients was associated with both defective LTP-like plasticity and reduced benefit of physical rehabilitation [44]. In patients with MS, physical exercise has been associated with acute and chronic increases of the levels of neurotrophins, such as brain-derived neurotrophic factor (BDNF) [45–47]. In animal models of MS, environmental enrichment and voluntary exercise promoted the expression of BDNF. This neurotrophin plays a well-documented role in LTP induction and in structural remodeling of dendritic spines [47]. As BDNF importantly modulates both inflammatory and neurodegenerative processes, the activity and expression of this molecule may significantly affect the disease course of MS [48].

Altered expression of neurotrophic factors has been reported in PMS phenotypes. A previous study reported lower CSF levels of platelet-derived growth factor (PDGF) in PMS patients compared to RR-MS patients and healthy subjects [9]. In the same study, PMS patients also showed absent LTP-like plasticity induced by the iTBS protocol. PDGF may have a neuroprotective effect in MS that prevents disability accumulation, by promoting tissue repair and survival and favoring LTP induction in vitro [9, 49, 50]. Interestingly, in RR-MS, higher CSF levels of PDGF at the time of diagnosis have been associated with a stable disease course and increased ability to compensate for new demyelinating lesions [51]. Therefore, increased neurodegeneration, chronic intrathecal inflammation and reduced expression of neurotrophins may contribute to disrupt LTP-like plasticity in PMS, causing an increase in progressive disability in these patients.

In this study, we confirmed a significantly impaired LTP-like plasticity in PMS patients compared with healthy subjects. In addition, we found that an 8-week physical rehabilitation program partially restored LTP-like plasticity expression. Accordingly, a significantly enhanced response to PAS was observed after rehabilitation

compared with baseline evaluation. It has been previously reported that 10 weeks of walking training significantly increased corticospinal excitability and was associated with higher MEP amplitudes and slope of recruitment curve, and decreased cortical silent period in the less affected hemisphere [52]. These data suggest that physical rehabilitation may represent a useful approach to improve synaptic plasticity expression in patients with PMS.

We found that rehabilitation is associated with reduced EDSS and FSS scores. The significant lower EDSS scores in PMS patients after rehabilitation could be explained by the inclusion of patients with early-stage progressive MS, who had low disability and had never undergone rehabilitation. Conversely, no significant differences emerged in T25FW and 9-HPT scores after rehabilitation. The lack of improvement in T25FW score, despite the reduced EDSS score, could possibly be explained by an overall increased endurance of walking, likely due to reduced fatigue, without affecting gait speed. In addition, the nonsignificant changes in 9-HPT scores after rehabilitation in PMS patients could be due to the large interindividual variability, as shown in Figure 3. Notably, when exploring correlations between LTP-like effect of PAS at baseline and improvement of clinical scores in PMS patients, a significant correlation was observed only with 9-HPT. Importantly, this result may suggest that LTP reserve could predict the effects of rehabilitation, in line with studies in RR-MS patients showing that LTP reserve correlated with clinical recovery [6]. Therefore, boosting synaptic plasticity may represent a potential therapeutic strategy to promote a stable disease course in PMS patients. The lack of a detailed evaluation of the rehabilitation protocol in PMS patients (including frequency, training time, type, volume, and progression) is a specific limitation of our study. This information is crucial to precisely define optimal exercise parameters to maximize the effects of rehabilitation on synaptic plasticity.

In this study, two different protocols were used to assess LTP-like plasticity in patients with RR-MS and PMS. Although this does not allow a direct comparison of the extent of plasticity, these protocols have been shown to induce comparable effects in healthy subjects [53] and have been widely used to explore LTP-like plasticity in patients with MS [26]; in addition, our study was specifically designed to investigate the effect of different motor activities on LTP-like plasticity in different MS phenotypes. Importantly, we tested LTP-like plasticity only in the intrinsic hand muscles (i.e., FDI and APB), thus being unable to assess the topographic specificity of a preferential training of the upper or lower limbs during both physical activity and rehabilitation. However, because the plastic effects are also evident in the cortical representations of muscles not involved in the training [52], having examined LTP-like plasticity in intrinsic hand muscles allowed us to investigate the widespread effects on CNS mechanisms involved in brain plasticity.

The lack of prospective TMS evaluation represents another important limitation of the present study, and further studies are needed to assess the long-term effects of preventive exercise and physical rehabilitation on synaptic plasticity. Follow-up data are equally important to evaluate the impact of preventive exercise and LTP reserve on MS disease course, to define the potential disease-modifying effect of exercise in both RR-MS and PMS.

Overall, the present study suggests that preventive exercise and rehabilitation may have a protective role against synaptic plasticity alteration in RR-MS and PMS patients and may represent a possible disease-modifying intervention by enhancing plasticity reserve and increasing the ability to compensate for ongoing neuronal damage.

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## CONFLICT OF INTEREST STATEMENT

Fabio Buttari acted as advisory board members for Teva and Roche and received honoraria for speaking or consultation fees from Merck Serono, Teva, Biogen Idec, Sanofi, and Novartis, and non-financial support from Merck Serono, Teva, Biogen Idec, and Sanofi. Diego Centonze is an advisory board member of Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva, and received honoraria for speaking or consultation fees from Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva. He is also the principal investigator in clinical trials for Bayer Schering, Biogen, Merck Serono, Mitsubishi, Novartis, Roche, Sanofi-Genzyme, and Teva. His preclinical and clinical research was supported by grants from Bayer Schering, Biogen Idec, Celgene, Merck Serono, Novartis, Roche, Sanofi-Genzyme and Teva. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, nor in the decision to publish the results. The other authors declare that they have no competing interests.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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