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Practice-dependent motor cortex plasticity is reduced in non-disabled multiple sclerosis patients

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HIGHLIGHTS

- Practice-dependent plasticity is reduced in early non-disabled RR-MS patients.
- M1 functional reorganization is impaired despite preserved early motor learning.
- Inflammation in MS could contribute to synaptic dysfunction.

ABSTRACT

Objectives: Skill acquisition after motor training involves synaptic long-term potentiation (LTP) in primary motor cortex (M1). In multiple sclerosis (MS), LTP failure ensuing from neuroinflammation could contribute to worsen clinical recovery. We therefore addressed whether practice-dependent plasticity is altered in MS.
Methods: Eighteen relapsing-remitting (RR)-MS patients and eighteen healthy controls performed 600 fast abductions of index finger in 30 blocks of 20 movements. Before and after practice, transcranial magnetic stimulation (TMS) was delivered over the hot spot of the trained first dorsal interosseous muscle. Movements kinematics, measures of cortical excitability, and the input/output curves of motor evoked potentials (MEPs) were assessed.
Results: Kinematic variables of movement improved with practice in patients and controls to a similar extent, although patients showed lower MEPs amplitude increase after practice. Practice did not change the difference in resting motor threshold values observed between patients and controls, nor did modulate short-interval intracortical inhibition. Clinical/radiological characteristics were not associated to practice-dependent effects.
Conclusions: Practice-induced reorganization of M1 is altered in non-disabled RR-MS patients, as shown by impaired MEPs modulation after motor learning.
Significance: These findings suggest that in RR-MS physiological mechanisms of practice-dependent plasticity are altered.

Abbreviations: ACC, peak acceleration; AMT, active motor threshold; CS, conditioning stimulus; cMAPs, compound muscle action potentials; EDSS, expanded disability status scale; FDI, first dorsal interosseous; fMRI, functional MRI; FSS, fatigue severity scale; I/O, input/output; ICF, intracortical facilitation; ISI, interstimulus interval; LTP, long-term potentiation; LST, lesion segmentation toolbox; M1, primary motor cortex; MEP, motor evoked potentials; MRI, magnetic resonance imaging; MS, multiple sclerosis; RMT, resting motor threshold; RR, relapsing-remitting; SICI, short-interval intracortical inhibition; SMA, supplementary motor area; TMS, transcranial magnetic stimulation; TS, test stimulus; T2Ln, total number of lesions; T2Lv, total lesion volume; VEL, peak velocity.

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1. Introduction

Synaptic activity at existing synapses could increase or decrease as a result of synaptic plasticity phenomena (Nelson and Turrigiano, 2008). Long-term potentiation (LTP) represents an enduring strengthening of synaptic efficacy followed by structural rearrangements, and constitutes one of the mechanisms underlying recovery after brain injury as well as learning and memory.

Both in animals and humans, motor training involves skill acquisition through synaptic LTP in primary motor cortex (M1) (Classen et al., 1998; Rioult-Pedotti et al., 1998; Muellbacher et al., 2001; Ziemann et al., 2004). In rats, motor training resulted in increased amplitude of field potentials in the M1 representation of the trained muscles (Rioult-Pedotti et al., 1998). Similarly, transcranial magnetic stimulation (TMS) studies investigating the effects of human motor learning on M1 functioning showed specific task-related modifications in M1 excitability and functional reorganization in the cortical representation of muscles involved in the task (Classen et al., 1998; Muellbacher et al., 2001).

Relapsing-remitting (RR)-MS represents a chronic inflammatory condition of the central nervous system characterized by acute inflammations and clinically stable periods. In MS, different proinflammatory and anti-inflammatory molecules involved in the induction and maintenance of the inflammatory process specifically modulate synaptic transmission and plasticity (Nisticò et al., 2013; Di Filippo et al., 2013; Mori et al., 2014a), influencing the possibility to compensate new brain lesions, and negatively affecting the disease course (Stampanoni Bassi et al., 2017).

Although it is fairly known that in MS inflammation is associated with altered TMS-induced LTP-like plasticity, practice-dependent plasticity has been not previously investigated. Increased knowledge about altered synaptic plasticity in MS patients could be relevant to better clarify whether central inflammation could hinder clinical recovery. Therefore, we explored in a group of early RR-MS patients whether practicing a repetitive simple motor task involves early motor learning, indexed as improvement in kinematic features of movement within practice, and triggers LTP in M1, comparing the results obtained in healthy controls.

2. Methods

2.1. Subjects

Eighteen RR-MS patients and 18 healthy control subjects participated in the study. The protocol was authorized by the ethics committee of IRCCS Neuromed, according to the Declaration of Helsinki. Written informed consent was obtained by each subject.

Patients were admitted to the Unit of Neurology of Neuromed Institute in Pozzilli (IS), Italy, and later diagnosed as suffering of RR-MS based on clinical, laboratory and magnetic resonance imaging (MRI) parameters, according to published criteria (Polman et al., 2011). The following clinical variables were collected. The first focal neurological deficit suspect for MS was considered as disease onset. Disease duration was measured as the time interval elapsing from disease onset to diagnosis (in months). Clinical relapses were considered as the occurrence of new or reappearance of specific symptoms for at least 24 h, independently of fever or infection. Clinical activity was defined as the presence of clinical relapse at the time of the study. The Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) was used by certified examining neurologists for assessing clinical disability. Fatigue was measured with the Fatigue Severity Scale (FSS) (Krupp et al., 1995). Inclusion criteria were: clinically definite diagnosis of RR-MS, absence of sensorimotor deficits in the upper limbs, EDSS < 3, and FSS score < 4. No immunoactive drug was given before or during the experimental paradigm and steroids or immunoactive therapies were started after.

2.2. Magnetic resonance imaging

Patients underwent brain and spine MRI (1.5 or 3.0 Tesla) before and after gadolinium. Radiological activity was defined as the presence of gadolinium-enhancing lesions at the time of the study. No cervical spinal cord lesions were detected in the patients enrolled. For each patient, the total number of lesions (T2Ln) and their relative total volume (T2Lv) was computed by using Lesion Segmentation Toolbox (LST) v. 2.0.15 (www. statistical-model ling.de/lst.html) for SPM (SPM12, MatLab v. 2015b; MathWorks, Natick, MA). Lesions were segmented using the Lesion Growth Algorithm (Schmidt et al., 2012) as implemented in the LST toolbox. The algorithm first segments the T1 images into three main tissue classes, namely cerebrospinal fluid, grey matter, white matter. This information is then combined with the coregistered FLAIR sequences to calculate lesion belief maps. By thresholding these maps with a pre-chosen initial threshold (κ), an initial binary lesion map is obtained which is subsequently grown along voxels that appear hyperintense in the FLAIR image. The result of this procedure is a lesion probability map. For all images, we used as threshold $\kappa = 0.3$.

2.3. Motor task and movement recordings

The motor task was adopted by Iezzi et al. (2010). All subjects were naïve to the task. Practice involved 600 fast abductions of the index finger with the dominant hand, executed in 30 blocks of 20 movements each. The hand position was constantly monitored throughout the task and participants were motivated to execute faster movements. To minimize fatigue, minimum intervals of 5-s between movements and 10-s between blocks were used. Movements were recorded using an optoelectronic device (BTS Bioengineering, Milan, Italy) with 12 infrared cameras (sampling rate, 120 Hz) able to follow in the three-dimensional space the displacement of reflective markers placed on the ulnar styloid process, on the head of 2nd and 5th metacarpal bones and on the distal phalanx of the 2nd finger. Ad-hoc algorithms were used to compute off-line peak velocity (VEL) and peak acceleration (ACC) of each movement. The kinematic parameters were averaged for each block. Motor learning was evaluated as the improvement in kinematic variables between the 1st and the 30th blocks.

2.4. Transcranial magnetic stimulation

Before and after practice, TMS was performed using Magstim 200^2 magnetic stimulators (The Magstim Company Ltd., Whitland, Dyfed, UK) connected to a figure-of-eight coil (external wing diameter, 9 cm) placed over the motor hot spot of the first dorsal interosseous (FDI) muscle of the dominant hand. For paired-pulse TMS, two separate Magstim 200^2 stimulators were connected to a Bistim^2 module (The Magstim Company).

Motor evoked potentials (MEPs) recorded from the FDI muscle were sampled at 5 kHz with a CED 1401 A/D interface (Cambridge Electronic Design, Cambridge, UK), amplified and bandpass filtered (20 Hz–1 kHz) with a Digitimer D360 (Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). Analysis was performed with Signal software (Cambridge Electronic Design).

Motor thresholds were calculated at rest (resting motor threshold, RMT) as the minimum stimulus intensity able to evoke MEPs with peak-to-peak amplitude $\geq$50 uV in five out of ten consecutive trials, and during voluntary contraction (active motor threshold, AMT) of the target muscle (approximately 20% of the maximum

voluntary contraction) as the lowest intensity able to elicit MEPs of ≥100 uV in five out of ten consecutive trials (Rossini et al., 2015).

In addition, we calculated the short-interval intracortical inhibition (SICI) and the intracortical facilitation (ICF) (Kujirai et al., 1993; Ilić et al., 2002). A conditioning stimulus (CS) with an intensity of 80% of the AMT was given before a test stimulus (TS) with an intensity to get MEPs of about 1 mV peak-to-peak in amplitude. For SICI the interstimulus interval (ISI) between the CS and the TS was 3 ms, whereas for ICF the ISI was 10 ms. We randomly delivered 30 stimulation trials (10 test pulses and 10 conditioned pulses at each ISI). To compensate for possible changes in MEPs size induced by practice, the intensity of the test pulse was adjusted after motor practice to evoke a test MEP of baseline size. Conditioned MEPs were expressed as percentage of the unconditioned MEPs.

Finally, we assessed the MEPs recruitment (input/output, I/O) curves by stimulating M1 at 110%, 130% and 150% of the RMT at baseline and 30, 45 and 60 minutes after practice. Twenty MEPs were recorded and averaged at each stimulation intensity for each time point. Changes in MEP size after motor practice (MEP post-/MEP pre-) were considered as an index of practice-induced LTP-like plasticity in M1.

2.5. Peripheral ulnar nerve stimulation

In a subgroup of 4 RR-MS patients and 8 healthy control subjects, the excitability of the ulnar nerve motor axons and spinal motoneurons pool innervating the FDI muscle was assessed before and 20 minutes after motor practice with electromyograph Dantec Keypoint (Natus Europe GmbH, Planegg, Germany). The ulnar nerve was stimulated at the wrist to obtain compound muscle action potentials (cMAPs) and F-waves from the relaxed FDI muscle of the dominant hand. To elicit cMAPs of maximal amplitude, the stimulation (cathode distal) was performed with single monophasic square-wave electric pulses (duration, 0.2 ms), of increasing intensity and randomly delivered (0.2–1 Hz). For the F-waves assessment, the ulnar nerve was stimulated (cathode proximal) with 20 supramaximal consecutive stimuli delivered at 1 Hz (duration, 0.2 ms; intensity, 20–30% above that used for the maximal cMAPs). Recordings were performed with electrodes placed over the FDI muscle (bandpass 20 Hz–3 kHz). Changes in cMAPs amplitude and in F-waves waves minimal latency, persistence (e.g., the percentage of measurable F-waves) and mean amplitude after motor practice were considered as indexes of motor axons and spinal motoneurons excitability modulation induced by practice.

2.6. Statistical analysis

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

Continuous variables were presented as mean (standard deviation, sd) or, if necessary, as median (25–75th percentiles). Categorical data were presented as frequency (percentage, %). Differences in continuous variables were evaluated by T test or, when necessary, by nonparametric Mann-Whitney test. Associations between two categorical variables were tested by Chi square test.

To evaluate changes in kinematic variables induced by practice in each subject, paired T test was applied comparing mean VEL and mean ACC between the 1st and the 30th block. Repeated measures ANOVA (RM-ANOVA) was performed assuming block (1st-30th) as within subjects factor and group (Controls, MS) as between subjects factor. Parametric Pearson correlation or nonparametric Spearman correlation was calculated to evaluate the association between changes in MEPs size after practice and clinical/radiological and demographic continuous variables.

To evaluate practice-induced changes in MEPs size, RM-ANOVA was applied assuming time (pre-post practice) as within subjects factor and group (Controls, MS) as between subjects factor. To evaluate differences in practice-induced MEPs changes between MS patients and Controls, separate RM-ANOVAs for each intensity (110%, 130% and 150%) were performed assuming time (baseline, post 30, post 45, post 60) as within subjects factor and group (Controls, MS) as between subjects factor. Post RM-ANOVA comparisons were performed using the T test with Bonferroni correction.

Possible effect of sex and age was also evaluated in the model. Possible changes in cMAPs amplitude and F-waves minimal latency, persistence and mean amplitude induced by motor task execution were evaluated by paired T test. For all analyses, $p < 0.05$ was considered statistically significant. All analyses were performed with IBM SPSS statistics for Windows, Version 20.0.

3. Results

Table 1 shows demographic and clinical/radiological characteristics of MS patients and Controls.

3.1. Practice-induced changes in kinematic variables

Improvement in kinematic variables between the 1st and the 30th block was considered as index of early motor learning. In each MS patient and in each Control subject a significant improvement of both ACC and VEL in the 30th block of movements was observed (Table 2 and Table 3). RM-ANOVA did not show a significant difference in VEL and ACC at the 30th block between MS patients and Controls (VEL: MS mean = 0.40, SE = 0.024 vs Controls mean = 0.039, SE = 0.024; Group effect: $F_{1,34} = 0.030$, $p = 0.864$. ACC: MS mean = 9.55, SE = 0.62 vs Controls mean = 9.60, SE = 0.62; Group effect: $F_{1,34} = 0.004$, $p = 0.952$) (Fig. 1). In the 1st block, VEL and ACC did not differ between controls and MS patients (VEL: MS mean = 0.343, sd = 0.095 vs Controls mean = 0.335, sd = 0.100; $p = 0.814$. ACC: MS mean = 7.87, sd = 2.378 vs Controls mean = 7.854, sd = 2.447; $p = 0.988$). No significant correlations emerged between age and improvement in kinematic variables (all $p > 0.2$). No significant correlations emerged between changes in kinematic variables and clinical/radiological characteristics (EDSS, disease duration, FSS, T2Ln, T2Lv) of MS patients (all $p > 0.2$).

3.2. Practice-induced changes in M1 excitability

TMS was well tolerated by all subjects and no adverse events were observed.

To assess practice-induced changes in M1 excitability we compared RMT, AMT, SICI and ICF before and after practice in the two groups. All M1 excitability measures did not correlate with age (all $p > 0.10$) and sex (all $p > 0.20$).

RM-ANOVA showed a significant overall effect of time meaning that practice significantly reduced RMT (pre mean = 47, SE = 1.07 vs post mean = 46.1, SE = 1.08; Time effect: $F_{1,34} = 19.756$, $p < 0.001$). The model showed an overall higher mean RMT in MS than in Controls (MS: mean = 49.6, SE = 1.62 vs Controls: mean = 43.6, SE = 1.41; Group effect: $F_{1,34} = 7.677$, $p = 0.009$), although difference between groups did not change after practice (Time × Group interaction effect: $F_{1,34} = 0.0096$, $p = 0.924$). Post hoc comparison showed that baseline RMT significantly differed between patients and controls (MS: mean = 51.5, SE = 1.64 vs Controls: mean = 44.8, SE = 1.57; $p = 0.009$), (Fig. 2A).

RM-ANOVA showed that overall AMT was significantly reduced after motor practice (pre mean = 438.6, SE = 1.23 vs post mean = 37.9, SE = 1.32; Time effect: $F_{1,34} = 6.300$, $p = 0.017$), with-

Table 1
Demographic and clinical characteristics of MS patients and Controls.

		MS n = 18	HC n = 18	p
Sex, F	n (%)	13 (72.2%)	9 (50%)	0.171
Age, years	mean (sd)	36.2 (5.43)	29.4 (5.91)	0.001
Disease duration, months	median (25–75th percentiles)	31 (6–50)		
EDSS	median (25–75th percentiles)	1 (0–2)		
Clinical activity	n (%)	0 (0%)		
Radiological activity	n (%)	4 (29%)		
T2 lesion number	mean (sd)	19.28 (9.75)		
T2 lesion volume, ml	mean (sd)	2.57 (1.89)		
FSS	median (25–75th percentiles)	2.1 (1.7–6)		

Abbreviations: EDSS, expanded disability status scale; FSS, fatigue severity scale; HC, healthy controls; MS, multiple sclerosis.

Table 2
Kinematic variables in control subjects.

Control subjects n.	VEL			ACC		
	1st mean (sd)	30th mean (sd)	p	1st mean (sd)	30th mean (sd)	p
1	0.485 (0.0558)	0.574 (0.0298)	<0.001	9.63 (2.0338)	14.26 (1.9122)	<0.001
2	0.389 (0.0574)	0.444 (0.0411)	0.001	9.51 (1.917)	10.62 (1.289)	0.020
3	0.364 (0.0615)	0.405 (0.0563)	0.083	8.46 (1.706)	10.4 (1.924)	<0.001
4	0.283 (0.0341)	0.406 (0.0414)	<0.001	6.74 (1.178)	9.99 (2.404)	<0.001
5	0.206 (0.0330)	0.401 (0.0612)	<0.001	3.83 (0.875)	5.59 (1.193)	<0.001
6	0.589 (0.0694)	0.678 (0.0604)	0.001	13.37 (3.232)	16.08 (2.114)	0.002
7	0.327 (0.0422)	0.674 (0.0426)	<0.001	8.92 (1.456)	19.27 (2.009)	<0.001
8	0.293 (0.0548)	0.412 (0.0533)	<0.001	6.32 (1.457)	11.86 (2.256)	<0.001
9	0.210 (0.021)	0.320 (0.0314)	<0.001	5.34 (0.932)	8.22 (1.128)	<0.001
10	0.444 (0.0447)	0.618 (0.0497)	<0.001	11.78 (1.378)	16.98 (2.064)	<0.001
11	0.235 (0.0421)	0.285 (0.0157)	<0.001	4.834 (1.253)	7.297 (0.765)	<0.001
12	0.237 (0.0377)	0.392 (0.022)	<0.001	5.362 (1.251)	10.328 (0.974)	<0.001
13	0.265 (0.0438)	0.352 (0.0402)	<0.001	6.25 (1.471)	8.814 (1.389)	<0.001
14	0.405 (0.0345)	0.443 (0.0377)	<0.001	9.82 (1.020)	12.165 (1.348)	<0.001
15	0.312 (0.0570)	0.496 (0.025)	<0.001	8.359 (1.684)	12.752 (1.436)	<0.001
16	0.319 (0.053)	0.381 (0.026)	0.001	7.383 (1.342)	10.518 (1.252)	<0.001
17	0.313 (0.019)	0.404 (0.022)	<0.001	7.456 (0.786)	9.102 (0.858)	<0.001
18	0.352 (0.0443)	0.385 (0.0366)	0.011	8.014 (1.374)	10.063 (1.718)	0.001
Mean	**0.335 (0.1001)**	**0.448 (0.1148)**		**7.854 (2.447)**	**11.349 (3.479)**	

Abbreviations: ACC, peak acceleration (m/s^2); VEL, peak velocity (m/s).

Table 3
Kinematic variables in MS patients.

MS subjects n.	VEL			ACC		
	1st mean (sd)	30th mean (sd)	p	1st mean (sd)	30th mean (sd)	p
1	0.44 (0.0447)	0.76 (0.0396)	<0.001	9.75 (1.412)	17.86 (1.928)	<0.001
2	0.28 (0.0492)	0.37 (0.0421)	<0.001	6.16 (1.572)	9.42 (1.98)	<0.001
3	0.537 (0.0582)	0.655 (0.025)	<0.001	9.71 (2.567)	13.17 (1.223)	<0.001
4	0.326 (0.0302)	0.398 (0.057)	<0.001	7.67 (1.519)	9.92 (1.511)	<0.001
5	0.516 (0.0196)	0.595 (0.0427)	<0.001	14.26 (1.336)	16.63 (2.430)	0.001
6	0.284 (0.0535)	0.452 (0.0513)	<0.001	5.87 (1.521)	10.22 (1.920)	<0.001
7	0.296 (0.0238)	0.331 (0.0256)	<0.001	5.71 (1.462)	7.05 (0.824)	<0.001
8	0.296 (0.0163)	0.348 (0.0223)	<0.001	6.33 (0.576)	8.51 (1.114)	<0.001
9	0.362 (0.0540)	0.555 (0.0280)	0.003	7.32 (1.447)	12.80 (1.297)	<0.001
10	0.363 (0.0413)	0.391 (0.0413)	0.022	8.56 (1.133)	10.130 (1.218)	<0.001
11	0.309 (0.041)	0.376 (0.019)	<0.001	7.37 (2.020)	9.32 (1.261)	0.001
12	0.229 (0.0309)	0.405 (0.0229)	<0.001	5.76 (0.819)	10.10 (1.486)	<0.001
13	0.409 (0.0255)	0.461 (0.0438)	<0.001	9.93 (1.260)	12.09 (1.027)	<0.001
14	0.372 (0.0272)	0.451 (0.0253)	<0.001	9.29 (1.045)	11.92 (0.908)	<0.001
15	0.399 (0.0332)	0.439 (0.0305)	0.001	8.854 (1.01)	11.094 (1.149)	<0.001
16	0.349 (0.035)	0.430 (0.0174)	<0.001	9.12 (1.321)	13.41 (1.462)	<0.001
17	0.189 (0.0312)	0.329 (0.0273)	<0.001	3.688 (0.986)	8.966 (0.830)	<0.001
18	0.211 (0.036)	0.392 (0.0707)	<0.001	6.254 (1.597)	9.556 (2.321)	<0.001
Mean	**0.343 (0.095)**	**0.452 (0.1172)**		**7.867 (2.3779)**	**11.231 (2.7683)**	

Abbreviations: ACC, peak acceleration (m/s^2); VEL, peak velocity (m/s).

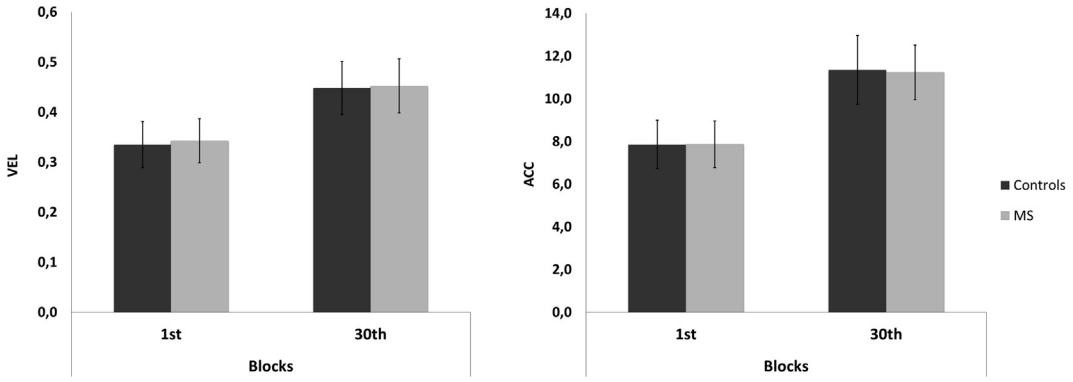

Fig. 1. Practice-induced changes in kinematic variables. Comparisons of VEL and ACC in the 1st and 30th block between MS patients and Controls. The error bars represent ±1.96 × SE. Abbreviations: ACC, peak acceleration (m/s^2); SE, standard error; VEL, peak velocity (m/s).

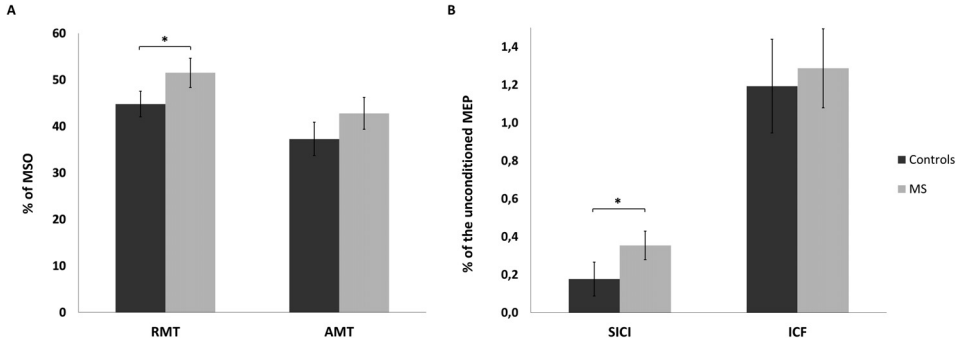

Fig. 2. Baseline motor cortex excitability measures. Comparison of AMT and RMT (A) and SICI and ICF (B) between MS patients and Controls. The error bars represent ±1.96 × SE. *p < 0.01. Abbreviations: AMT, active motor threshold; ICF, intracortical facilitation; MEP, motor evoked potential; MSO, maximum stimulator output; SICI, short-interval intracortical inhibition; RMT, resting motor threshold; SE, standard error.

out differences between the two groups (Group: $F_{1,34}$ = 2.709; p = 0.109). Post hoc comparison showed that baseline AMT did not differ between patients and Controls (MS: mean = 42.8, SE = 1.82 vs Controls: mean = 37.3, SE = 1.35; p = 0.09), (Fig. 2A).

Considering intracortical excitability, baseline SICI significantly differed between MS patients and Controls, in particular, MS patients showed reduced SICI compared to Controls (MS mean = 0.35, SE = 0.038 vs Controls mean = 0.18, SE = 0.045; Group effect ($F_{1,34}$ = 8.241, p = 0.007), (Fig. 2B). Moreover, SICI was not significantly modified by practice (Time effect: $F_{1,34}$ = 0.246, p = 0.623). ICF did not significantly differ between MS patients and Controls (Group effect: $F_{1,34}$ = 0.317, p = 0.577) and no changes were evidenced after motor practice (Time effect: $F_{1,34}$ = 1.002, p = 0.324), (Fig. 2B).

In MS patients, no significant correlations emerged between M1 excitability measures and clinical/radiological characteristic (all p > 0.20).

3.3. Practice-induced LTP-like plasticity in M1

No effect of age and sex on I/O curves was evidenced in the RM-ANOVAs (all p > 0.1).

Considering 110% RMT stimulus intensity, RM-ANOVA showed a significant effect of time (Time effect: $F_{3,102}$ = 51.678, p < 0.001). Overall, a significant increase of MEPs size was observed at all time points: post 30 (mean = 1.4, 95% CI = 1.3 to 1.5; p < 0.001), post 45 (mean = 1.5, 95% CI = 1.4 to 1.6; p < 0.001), and post 60 (mean = 1.57, 95% CI = 1.5 to 1.7; p < 0.001). A significant difference was also observed between MS patients and Controls (Group effect: $F_{1,34}$ = 45.297, p < 0.001). In particular, after practice, Controls showed higher MEPs size compared with MS patients (Controls: mean = 1.57, 95% CI = 1.5 to 1.6, MS: mean = 1.16, 95% CI = 1.1 to 1.3; p < 0.001). In addition, a significant interaction Time × Group was found ($F_{3,102}$ = 15.275, p < 0.001). Specifically, post hoc comparisons showed that in Controls a significant increase of MEPs size was observed at each time point (all p < 0.001). Conversely, in MS patients a significant increase was observed post 45 (mean = 1.2, 95% CI = 1.1 to 1.4; p = 0.018) and post 60 (mean = 1.3, 95% CI = 1.1 to 1.4; p = 0.005) and no significant differences emerged post 30 (mean = 1.1, 95% CI = 1 to 1.3; p = 0.186), (Fig. 3A).

Considering 130% RMT stimulus intensity, the RM-ANOVA model showed a significant time effect (Time effect: $F_{3,102}$ = 69.092, p < 0.001), MEPs amplitude showed a significant increase at all time intervals: post 30 (mean = 1.4, 95% CI = 1.3 to 1.5; p < 0.001), post 45 (mean = 1.5, 95% CI = 1.4 to 1.6; p < 0.001) and post 60 (mean = 1.5, 95% CI = 1.4 to 1.6; p < 0.001). A significant difference between MS patients and Controls was observed (Group effect: $F_{1,34}$ = 35.955, p < 0.001). Overall, Controls showed higher MEPs size (mean = 1.6, 95% CI = 1.5 to 1.7) compared with MS patients (mean = 1.2, 95% CI = 1.1 to 1.3). A significant Time × Group interaction was found ($F_{3,102}$ = 22.783, p < 0.001). In particular, post hoc comparisons showed a significant increase of MEPs amplitude at each time point in Controls: post 30 (mean = 1.6, 95% CI = 1.5 to 1.7; p < 0.001), post 45 (mean = 1.8, 95% CI = 1.7 to 1.9; p < 0.001) and post 60 (mean = 1.9, 95% CI = 1.7 to 2.0; p < 0.001). Conversely, in MS patients MEPs size significantly increased at post 30 (mean = 1.2, 95% CI = 1.1 to 1.4; p = 0.005) and at post 45 (mean = 1.3, 95% CI = 1.1 to 1.4; p = 0.001), whereas no significant differences were observed at post 60 (mean = 1.2, 95% CI = 1.0 to 1.3; p = 0.103), (Fig. 3B).

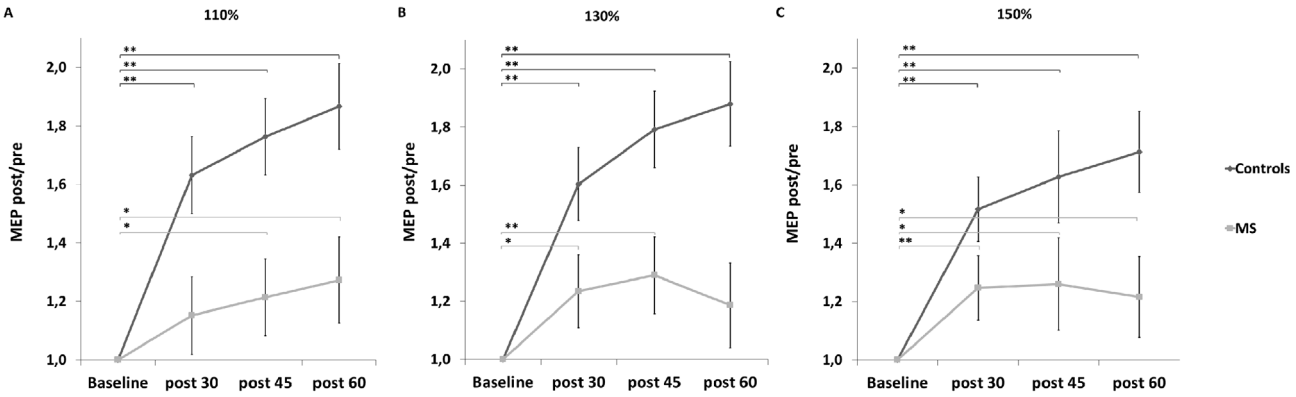

Fig. 3. Practice-induced modulation of MEPs amplitude. I/O curves at 110% (A), 130% (B) and 150% (C) of RMT at different time points after practice in the two groups. Y axis represents the ratio between the mean MEP amplitude post-practice and the mean MEP amplitude at baseline. X axis represents the time course of LTP-like effect. The error bars represent ±1.96 × SE. ** $p < 0.01$ * $p < 0.05$. Abbreviations: I/O curves, input/output curves; LTP, long-term potentiation; MEP, motor evoked potential; RMT, resting motor threshold; SE, standard error.

At 150% RMT stimulus intensity, a significant effect of time was observed (Time effect: $F_{3,102} = 49.090$, $p < 0.001$). In particular, a significant increase of MEPs size was observed at all time points tested: post 30 (mean = 1.4, 95% CI = 1.3 to 1.5; $p < 0.001$), post 45 (mean = 1.4, 95% CI = 1.3 to 1.6; $p < 0.001$), and post 60 (mean = 1.5, 95% CI = 1.4 to 1.6; $p < 0.001$). Moreover, Controls showed higher increase of MEPs amplitudes compared with MS patients (Controls mean = 1.5, 95% CI = 1.4 to 1.6; MS mean = 1.2, 95% CI = 1.1 to 1.3; $F_{1,34} = 18.179$; $p < 0.001$). A significant Time × Group interaction was found ($F_{3,102} = 11.497$, $p < 0.001$). Post hoc comparisons showed that MEPs size increased at each time point in Controls (post 30 mean = 1.5, 95% CI = 1.4 to 1.6; post 45 mean = 1.6, 95% CI = 1.5 to 1.8; post 60 mean = 1.7, 95% CI = 1.6 to 1.9; all $p < 0.001$). Also in MS patients a significant increase was observed at post 30 (mean = 1.2, 95% CI = 1.1 to 1.4; $p = 0.001$), at post 45 and at post 60 (post 45 mean = 1.3, 95% CI = 1.1 to 1.4, $p = 0.016$; post 60 mean = 1.2, 95% CI = 1.1 to 1.4; $p = 0.027$) (Fig. 3C).

Finally, no significant correlations were observed between practice-induced plasticity, clinical parameters (EDSS, disease duration, FSS), radiological measures (T2Ln and T2Lv) and neurophysiological data (I/O and SICI at baseline) (all $p > 0.20$).

3.4. Practice-induced changes in ulnar motor axons and spinal cord excitability

To assess practice-induced influence on ulnar motor axons and motor spinal pool excitability, we compared FDI cMAPs amplitude and F-waves minimal latency, persistence and mean amplitude before and after practice. No significant changes emerged in all measures explored after practice (all $p > 0.20$).

4. Discussion

Physical rehabilitation promotes clinical recovery after neurological damage through practice-dependent LTP. In rats, after focal brain ischemia LTP could represent the mechanism underlying clinical recovery (Centonze et al., 2007). Accordingly, physical exercise induces significant reorganization in surviving neurons such as increased synaptic remodeling and LTP (Biernaskie and Corbett, 2001). Also in humans, the LTP-like effect induced by TMS, the so-called "LTP reserve", measured shortly after brain damage correlated with clinical recovery in stroke and in MS patients (Di Lazzaro et al., 2010; Mori et al., 2014b).

In the present study we found that, in a group of early non-disabled RR-MS patients, MEPs amplitude modulation by practicing a simple repetitive motor task is reduced in presence of preserved practice-induced early motor learning, as demonstrated by the significant improvement of kinematic variables at the end of motor performance. The finding that MS patients displayed normal early motor learning agrees with previous studies demonstrating preserved online motor learning tested with different motor tasks (Zeller et al., 2010; Tomassini et al., 2011; Rumpf et al., 2018). Despite the variability in MEPs amplitudes after motor practice may depend on the relatively small number of patients, our results are in line with previous evidence that in RR-MS patients TMS-induced plasticity is altered (Mori et al., 2011; 2012; 2014a). In particular, LTP-like plasticity induced by the intermittent theta-burst stimulation was reduced during MS relapses (Mori et al., 2011), and could be restored after six-months treatment with interferon beta-1a (Mori et al., 2012). In stable RR-MS patients central inflammation levels correlated with altered TMS-induced plasticity (Mori et al., 2014a), also supporting the role of silent CSF inflammation in promoting alterations of synaptic functioning. In our study, the presence of only 4 patients with radiological disease activity did not allow to perform subgroup analysis and explore whether active and silent inflammation could differently affect practice-induced plasticity. This limitation represents an issue deserving further investigations.

Early motor learning typically develops after a single practice session of sufficient duration (Classen et al., 1998). Indeed, motor learning mainly evolves through two stages: an early phase characterized by fast improvement within practice, and a subsequent slower phase in which further improvement develops across additional practice sessions (Dayan and Cohen, 2011). In humans, studies with positron emission tomography and functional MRI (fMRI) have shown that as practice progresses early motor learning is associated with reduced activation of dorsolateral prefrontal cortex, M1 and pre-supplementary motor area (pre-SMA) (Floyer-Lea and Matthews, 2005), while activity increases in the premotor cortex, SMA, parietal regions, striatum and the cerebellum (Grafton et al., 2002; Floyer-Lea and Matthews, 2005). It has been suggested that increased activation may reflect recruitment of additional cortical areas during practice, whereas decreased activity could be related to changes in neuronal functional characteristics optimized to task performance as learning progresses (Poldrack, 2000). Previous TMS studies showed that human motor learning involves changes in M1 excitability, indicating some reorganization of motor outputs (Muellbacher et al., 2001) and the induction of LTP-like plasticity within M1 (Ziemann et al., 2004). In our study, the lack of F-waves changes after early motor learning, although tested in a small sample, is in line with the possibility that motor

practice has induced changes in M1 representation of the trained muscle. Indeed, F-waves represent a sensitive marker of motoneurons excitability (Peioglou-Harmoussi et al., 1985). This observation may support the idea that the reported effects have occurred at cortical level likely involving LTP-like plasticity. Although spinal cord excitability could be assessed evaluating the H reflex, it is worth to note that H reflexes are commonly difficult to obtain in hand muscles (Pierrot-Deseilligny and Mazevet, 2000).

Several possible mechanisms could explain the lack of practice-induced LTP observed in our patients. The possibility that motor impairment may have altered movement execution preventing LTP from developing is unlikely, as only patients with mild functional involvement and without cervical spinal cord damage were included, and motor performance was comparable between patients and controls. In addition, clinical and radiological characteristics were not associated to practice-dependent effects. Motor learning triggered by repeating simple movements has been related to reduced SICI (Rosenkranz et al., 2007; Coxon et al., 2014), heralding a relationship between GABA levels in M1 and motor learning although with some exceptions (Rogasch et al., 2009; Rosenkranz and Rothwell, 2006). The possibility that our patients displayed lower LTP-like effect after motor practice due to different modulation of SICI is unlikely, as both in patients and controls SICI did not significantly change after motor practice. Moreover, whether different levels of baseline cortical excitability could influence subsequent modulation of MEP after motor practice is uncertain, as no significant correlations emerged between both I/O curves and SICI at baseline and practice-induced changes in MEPs.

As fatigue represents a frequent symptom in MS patients, previous TMS studies explored the neurophysiological correlates of fatigue, in particular MS patients with fatigue showed less SICI compared to MS patients without fatigue and Controls (Liepert et al., 2005). The presence of fatigue was carefully assessed, and all patients included showed no significant levels of fatigue as measured by the FSS (Krupp et al., 1995). Furthermore, our motor task was specifically designed to minimize the occurrence of fatigue accordingly, participants were allowed to fully recover between movements and blocks being intervals individually adjusted. In addition, as no significant correlations emerged between FSS score and both motor performance and practice-induced plasticity, it is improbable that fatigue could account for the lack of MEPs modulation after practice in our cohort of patients.

To explain the present findings, one possibility could be that, differently from healthy subjects, improvement of motor performance in MS patients may have occurred through a different recruitment pattern of synergistic and antagonistic muscles which could have possibly contributed to the lack of MEP modulation after practice. Alternatively, our patients could have improved the kinematic variables during practice through an abnormally rapid activation of brain areas other than M1, thus explaining the subsequent lacking M1 plasticity induced by practice. Functional imaging studies evidenced that motor learning modulates the activity in M1 and in a large subset of functionally connected regions (Poldrack, 2000). In particular, task-based fMRI studies previously reported that early, non-disabled, RR-MS patients showed widespread activation compared to healthy controls in different regions, including for example the dorsal premotor cortex and the SMA (Morgen et al., 2004; Rocca et al., 2005). The functional significance of brain overactivation in MS has not been elucidated, in particular, increased activation could represent either the effect of compensatory mechanisms (Filippi and Rocca, 2013), or an epiphenomenon of disease (Lenzi et al., 2007). Accordingly, it has been evidenced that diffused fMRI activation correlated with structural damage, suggesting that alternative mechanisms (i.e. loss of transcallosal inhibition) could be involved (Lenzi et al., 2007).

In the present study, impaired practice-induced LTP-like plasticity in M1 of RR-MS patients in presence of preserved early motor learning agrees with the hypothesis that recruiting additional brain regions could be useful to optimize motor performance in early RR-MS. Nevertheless, the lack of functional imaging studies in our sample of subjects is a limitation that cannot allow us to draw definitive conclusions and therefore further studies combining TMS with fMRI are needed to clarify the role of MRI overactivation.

5. Conclusions

Our results extend previous evidence showing that also practice-induced plasticity is altered in non-disabled, clinically stable, early RR-MS patients. These findings suggest that inflammation in MS could represent a deleterious factor contributing to synaptic dysfunction.

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Declaration of Competing Interest

The Authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: F.B. is an Advisory Board member of Teva and Roche, he received honoraria for speaking and travel grants from Merck Serono, Teva, Biogen, Sanofi-Genzyme, and Novartis. F.S. is an Advisory Board member of Merck Serono and Novartis. D.C. is an Advisory Board member of Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Genzyme, Teva and received honoraria for speaking or consultation fees from Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Genzyme, Teva. He is also the principal investigator in clinical trials for Bayer Schering, Biogen, Merck Serono, Mitsubishi, Novartis, Roche, Sanofi-Genzyme, Teva. His preclinical and clinical research was supported by grants from Bayer Schering, Biogen Idec, Celgene, Merck Serono, Novartis, Roche, Sanofi-Genzyme and Teva. M.S.B., P.M., N.D.P., A.S., L.G., L.P., G.P., I.S., R.F., P.B. and E.I. have no disclosures.

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