

Cortical excitability changes over time in progressive multiple sclerosis

Samar S. Ayache, MD, PhD^{a,b}
Alain Créange, MD, PhD^{a,e}
Wassim H. Farhat, MD^{a,b,e}
Hela G. Zouari, MD^{a,b,c}
Catherine Lesage, BSc^e
Ulrich Palm, MD^{a,d}
Mohammed Abdellaoui, MD^e
Jean-Pascal Lefaucheur, MD, PhD^{a,b}

^a Faculté de Médecine de Créteil, Université Paris Est Créteil, EA 4391, Créteil, France

^b Hôpital Henri-Mondor, AP-HP, Service de Physiologie, Explorations Fonctionnelles, Créteil, France

^c CHU Habib Bourguiba, Service d'Explorations Fonctionnelles, Sfax, Tunisia

^d Ludwig-Maximilian University, Department of Psychiatry and Psychotherapy, Munich, Germany

^e Hôpital Henri-Mondor, AP-HP, Service de Neurologie, Créteil, France.

Correspondence to: Jean-Pascal Lefaucheur
E-mail: jean-pascal.lefaucheur@hmn.aphp.fr

Summary

In 25 patients with progressive forms of multiple sclerosis (MS), motor cortex excitability was longitudinally studied over one year by means of transcranial magnetic stimulation (TMS). The following TMS parameters were considered: resting and active motor thresholds (MTs), input-output curve, short-interval intracortical inhibition (SICI), and intracortical facilitation. Clinical evaluation was based on the Expanded Disability Status Scale (EDSS).

In the 16 patients not receiving disease-modifying drugs, the EDSS score worsened, resting MT increased, and SICI decreased. By contrast, no clinical or neurophysiological changes were found over time in the nine patients receiving immunomodulatory therapy.

The natural course of progressive MS appears to be associated with a decline in cortical excitability of both pyramidal neurons and inhibitory circuits. This pilot study based on a small sample suggests that disease-modifying drugs may allow cortical

excitability to remain stable, even in patients with progressive MS.

KEY WORDS: cortical excitability, disease course, motor cortex, multiple sclerosis, progressive form, treatment.

Introduction

Multiple sclerosis (MS) is a common neurological disease and the leading cause of disability in young adults (Sadovnick and Ebers, 1993). Its natural history, which can include recurrent relapses or progressive neurological deterioration, is highly variable among individuals (Confavreux et al., 2003). In some patients, the disease remains stable or progresses only mildly over many years. In others, it rapidly reaches a severe and irreversible stage. It is thought that once the disease has become established its progression cannot be modified, and that no therapeutic options exist that can stop or reverse progressive forms of MS. Patients with primary or secondary progressive MS usually face an irreversible decline in their functional capacity (Koch et al., 2013; Rice et al., 2013). In the context of therapeutic trials focusing on progressive forms of MS, one of the main challenges is to define predictive criteria that could objectively assess the impact of treatment on the disease course. Transcranial magnetic stimulation (TMS) of the brain is a non-invasive tool widely applied for various therapeutic purposes in neurological and psychiatric diseases (Lefaucheur et al., 2014). In addition, single- and paired-pulse TMS paradigms can provide information on motor cortex excitability and plasticity, and thus make it possible to better understand underlying pathophysiological mechanisms and to evaluate the efficacy of therapeutic strategies (Maeda and Pascual-Leone, 2003; Badawy et al., 2012). The aim of our study was to apply TMS techniques to monitor motor cortex excitability changes in progressive MS.

Materials and methods

Patients

During a study lasting two years, 34 MS patients (18 women and 16 men, aged from 29 to 75 years, mean: 57 years) were enrolled from the Neurology

Department of the Henri Mondor Hospital, according to the following inclusion criteria: i) confirmed diagnosis of progressive MS according to the revised McDonald's criteria (Polman et al., 2011); ii) no other neurological disease; iii) signature of the informed consent; iv) no absolute contraindication to TMS (no ferromagnetic implants and no history of epilepsy) (Rossi et al., 2009); v) presence of motor evoked potentials (MEPs) recordable from the muscles of at least one hand. The present study was approved by our Institutional Review Board. Patients were classified as having primary (n=16) or secondary (n=18) progressive MS and they had a disease duration ranging from one to 45 years (mean: 20 years).

Three evaluations, at three time points: T1 (baseline), T2 and T3, were performed in each patient, with six-month inter-evaluation intervals, i.e. corresponding to one year of follow-up. Each evaluation consisted of a neurological examination and a neurophysiological investigation, which was led by the same investigator (SSA).

Nine patients did not complete the study for various reasons: loss to follow-up (n=6), withdrawal of consent (n=2), or death unrelated to the study (n=1). The 25 patients who completed the study were classified into two groups according to their MS treatment. Group 1 consisted of nine patients treated with interferon beta 1b (n=2), glatiramer acetate (n=1), monthly intravenous infusion of methylprednisolone (n=2), methotrexate (n=3), or natalizumab (n=1). Group 2 consisted of 16 patients who did not receive disease-modifying drugs.

Neurological examination

At each visit, a detailed medical history and data from standard neurological examination were recorded and the patient's disability status was scored using the Expanded Disability Status Scale (EDSS). Particular attention was paid to the occurrence of any new neurological deficits or the deterioration of a pre-existing neurological disorder.

Neurophysiological investigation

The following TMS parameters of motor cortex excitability were studied: i) resting motor threshold (rMT); ii) active motor threshold (aMT); iii) input-output curve (IOC) in two conditions: at rest (rIOC) and during voluntary muscle contraction (aIOC); iv) short-interval intracortical inhibition (SICI) and v) intracortical facilitation (ICF).

We recorded MEPs from the first dorsal interosseous (FDI) muscle using a pair of adhesive pre-gelled surface electrodes (Ref. 9013S0242, Natus-Dantec, Skovlunde, Denmark). Recordings were performed on the most severely affected limb or hemibody and repeated on the same side upon subsequent evaluations. In MS patients equally affected on both sides, recordings were taken from the right FDI muscle.

Electromyographic (EMG) signals were amplified (50-500 μ V/division) and filtered (20 Hz-2 kHz), and then stored in a laboratory computer (Phasis II machine; EsaOte, Florence, Italy) for later off-line analysis. Complete FDI muscle relaxation was ensured via auditory feedback and looking for the absence of background activity on the screen of the EMG device (except for aMT and aIOC measurement during which the muscle was maintained at a stable level of voluntary contraction against moderate resistance).

Cortical excitability studies were performed using one Magstim 200 stimulator (Magstim Co., Carmarthen, Wales, UK) for rMT, aMT, rIOC and aIOC recordings, and two Magstim 200 stimulators connected via a Bistim module (Magstim Co.) to deliver paired pulses for SICI and ICF recordings. Patients were seated in a comfortable armchair; they had a cap placed on their head, and the head was kept still throughout recordings. A 90-mm circular coil (P/N 9784-00, Magstim) was used and centered on the vertex, whose position was defined according to anatomical landmarks of the skull. The coil was applied on the appropriate side (A or B), with the handle pointing backwards, to stimulate preferentially the motor cortex contralateral to the side of recording (Ayache et al., 2014). In addition, the position of the coil was marked on the cap to optimize coil repositioning during subsequent tests.

RESTING AND ACTIVE MOTOR THRESHOLDS

The rMT and aMT were defined as the minimal stimulus intensity required to evoke, in a series of 10 trials, five MEPs with the required peak-to-peak amplitude, set at 50 μ V and 200 μ V respectively. A step width of 1% of maximum stimulator output was used for this purpose.

INPUT-OUTPUT CURVES

Once the rMT and aMT had been determined, the rIOC and aIOC were studied by gradually increasing the stimulation intensity from 110% to 140% of rMT and aMT, respectively, by steps of 10%. At each level of stimulation intensity, four trials were performed and averaged, and the mean peak-to-peak MEP amplitude was calculated. The slope of each IOC was determined at its most linear part (Lefaucheur et al., 2012). In addition, maximal peak-to-peak MEP amplitude and minimal MEP latency measured on maximal MEPs were retained for analysis.

SHORT-INTERVAL INTRACORTICAL INHIBITION AND FACILITATION

A paired-pulse TMS paradigm was used to assess the parameters SICI and ICF with the interstimulus interval (ISI) set at 2, 3 and 4 ms for SICI and at 10, 12 and 15 ms for ICF. Conditioning and test stimulation inten-

sities were set at 80% and 120% of rMT, respectively. Eight non-conditioned single test stimuli and four paired stimuli at each ISI were delivered and the recorded values were averaged. In each ISI condition, the mean peak-to-peak MEP amplitude to paired stimuli was calculated and expressed as the percentage of the test MEP amplitude (p/t MEP%). The amount of inhibition or facilitation was determined according to the following formula: SICI= 100%–p/t MEP% and ICF= p/t MEP% –100%. Mean and maximum SICI and ICF values were retained for analysis (Kujirai et al., 1993; Lefaucheur et al., 2006, 2012).

Data analysis

Descriptive statistics were expressed as mean ± standard deviation and analyses were performed using InStat 3 (GraphPad Software, San Diego, CA, USA). Non-parametric tests were applied, since not all data passed the Kolmogorov-Smirnov normality test. Demographic data and neurophysiological results at baseline were compared between the patients in group 1 (treated) and group 2 (untreated) using the Mann-Whitney test for quantitative data and Fisher’s exact test for categorical data. Correlations between age or

EDSS scores and neurophysiological parameters at baseline were assessed using the Spearman test.

In the whole set of patients, data obtained at the three time points were compared using the Friedman test (repeated measures ANOVA). A p-value of less than 0.05 was considered significant. In the event of significance, Dunn’s multiple comparisons test was performed. Correlations between EDSS scores and any significantly modified neurophysiological parameter were assessed using the Spearman test.

Results

Baseline comparisons

Group 1 (9 patients receiving treatment) comprised four women and five men, aged between 35 and 74 years (mean: 50.9 years). They had primary (n=5) or secondary (n=4) progressive MS, a disease duration ranging from two to 28 years (mean: 14.8 years) with a progressive phase duration ranging from one to 25 years (mean: 9.6 years), and EDSS scores at baseline ranging from 4 to 7.5 (mean: 5.8) (Table I).

Group 2 (16 patients not receiving treatment) comprised nine women and seven men, aged between 29

Table I - Mean values (± SD) of EDSS scores and cortical excitability parameters obtained in treated and untreated patients at the three time points.

		T1	T2	T3	p-value	
EDSS score	Treated	5.8 ± 1.2	5.9 ± 1.2	5.9 ± 1.2	0.1353	
	Untreated	6.5 ± 1.5	6.5 ± 1.5	6.6 ± 1.6	0.0224	
RMT (%)	Treated	69.9 ± 13.3	68.4 ± 13.5	69 ± 10.9	0.1590	
	Untreated	70.3 ± 15.3	75.8 ± 15.9	75.7 ± 13.7	0.0115	
Minimal resting MEP latency (ms)	Treated	29.2 ± 4.9	29.7 ± 4.8	28.4 ± 4.6	0.5488	
	Untreated	26.6 ± 2.9	27.4 ± 4.4	29.2 ± 8.2	0.2139	
Maximal resting MEP amplitude (µV)	Treated	890.2 ± 832.6	653.6 ± 792.2	910.8 ± 997.8	0.3281	
	Untreated	361.4 ± 539.5	459.2 ± 713.1	616.8 ± 1119.9	0.9355	
Resting MEP recruitment curve (slope)	Treated	23.5 ± 23.1	12.3 ± 12.4	26.4 ± 28.7	0.3553	
	Untreated	13.0 ± 20.7	15.5 ± 20.2	27.8 ± 48.5	0.3281	
Active motor threshold (%)	Treated	65.1 ± 11.9	63.4 ± 13.9	67 ± 10.1	0.9155	
	Untreated	69.3 ± 15.7	73.9 ± 18.4	73.1 ± 17.6	0.5379	
Minimal active MEP latency (ms)	Treated	27.8 ± 5.1	28.4 ± 5.5	27.9 ± 4.3	0.9999	
	Untreated	25.6 ± 3.2	27.1 ± 4.2	28.2 ± 7.3	0.4233	
Maximal active MEP amplitude (µV)	Treated	1472.3 ± 1131.9	1496.6 ± 1745.1	943.2 ± 669.2	0.3977	
	Untreated	996.1 ± 1770.4	729.0 ± 842.9	715.6 ± 718.6	0.9832	
Active MEP recruitment curve (slope)	Treated	31.8 ± 32.0	46.2 ± 58.1	23.2 ± 21.0	0.1916	
	Untreated	27.7 ± 55.6	26.4 ± 28.7	23.2 ± 29.3	0.2851	
SICI (%)	Mean value	Treated	37.1 ± 32.0	30.9 ± 67.3	31.5 ± 48.1	0.9712
		Untreated	60.6 ± 24.5	30.2 ± 34.0	27.0 ± 46.8	0.0063
	Maximal value	Treated	54.2 ± 36.4	64.4 ± 32.5	65.1 ± 21.2	0.8135
		Untreated	76.7 ± 17.3	59.1 ± 20.6	57.3 ± 20.9	0.0171
Intracortical facilitation (%)	Mean value	Treated	64.3 ± 119.5	49.8 ± 65.8	64.0 ± 123.5	0.6854
		Untreated	87.5 ± 76.8	76.3 ± 88.8	107.9 ± 168.6	0.7659
	Maximal value	Treated	104.7 ± 146.1	96.9 ± 107.3	132.4 ± 208.4	0.6854
		Untreated	151.5 ± 124.7	122.1 ± 122.1	156.3 ± 209.2	0.7659

Abbreviations: EDSS=Expanded Disability Status Scale; RMT=resting motor threshold; MEP: motor evoked potential; SICI=short-interval intracortical inhibition. Significant p-values are in bold and underlined.

and 75 years (mean: 59.3 years). They had primary (n=9) or secondary (n=7) progressive MS, a disease duration ranging from one to 45 years (mean: 22.5 years) with a progressive phase duration ranging from one to 31 years (mean: 15.7 years), and EDSS scores at baseline ranging from 2.5 to 8 (mean: 6.5) (Table I).

The patients in group 1 were younger than those in group 2 (50.9±11.1 years vs 59.3±10.6 years, p=0.0292, Mann-Whitney test). Conversely, there was no difference between the groups in gender (p=0.6882, Fisher's test), ratio between primary and secondary progressive MS form (p=1.0), disease duration (p=0.1929, Mann-Whitney test), progressive phase duration (p=0.1335), or EDSS scores (p=0.1324). The neurophysiological results, too, were not found to differ between the two groups (p-values ranging between 0.1195 and 0.6505), with the exception of active MEP amplitude, which was higher in group 1 than in group 2 (1472.3±1131.9 μV vs 996.1±1770.4 μV, p=0.0428).

In the complete series of patients, age did not correlate with any of the neurophysiological parameters (p-values ranging between 0.2224 and 0.9679, Spearman test). By contrast, EDSS scores correlated negatively with resting MEP amplitude (r=-0.4704, p=0.0176) and tended to correlate negatively with active MEP amplitude (r=-0.3818, p=0.0597) and aIOC (r=-0.4194, p=0.0584) and positively with rMT (r=0.3653, p=0.0725) and aMT (r=0.3596, p=0.0774). There was no correlation between EDSS scores and the other neurophysiological parameters (p-values ranging between 0.1061 and 0.5595).

Follow-up study

In the follow-up study, EDSS scores increased only in group 2 (untreated patients) (Friedman test, p<0.05) (Table I), significantly at T3 compared to T1 (Dunn's post-test, p<0.05) (Fig. 1). In group 1 (treated

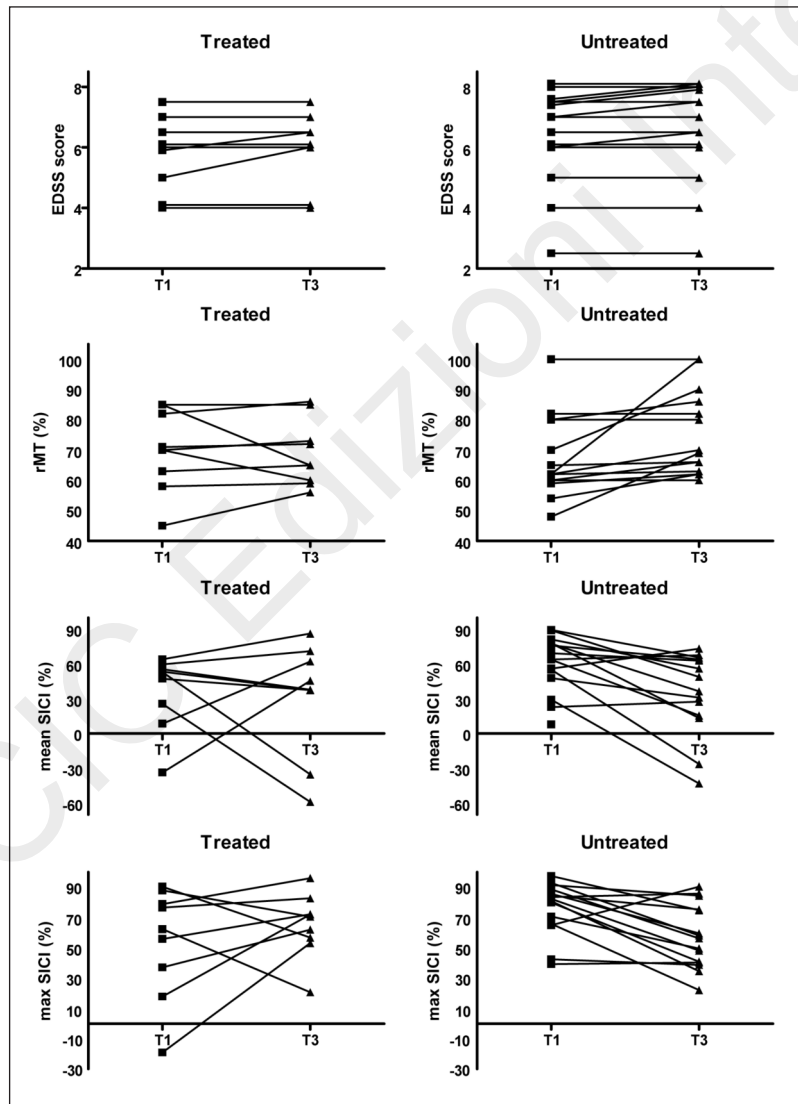


Figure 1 - Individual changes over time in EDSS scores, resting motor threshold (rMT), and short-interval intracortical inhibition (SICI) in treated and untreated patients. Changes were statistically significant in untreated patients (EDSS and rMT increased and mean and max SICI decreased), but not in treated patients.

patients), EDSS score changes did not reach statistical significance.

All the patients completed the study with recordable MEPs. In the follow-up study, rMT increased and SICI decreased (mean and maximal values) only in group 2 (untreated patients) (Friedman test, $p < 0.05$) (Table I). The other parameters, including maximal MEP amplitude and minimal MEP latency, did not change. Dunn's multiple comparison post-test showed a significant increase in rMT and decrease in maximal SICI at T3 compared to T1, as well as a decrease in mean SICI at both T2 and T3 compared to T1 ($p < 0.05$) (Fig. 1). These changes did not correlate with EDSS score changes observed in the same group (p-values ranging from 0.23 to 0.82, Spearman test). In group 1 (treated patients), there was no change in cortical excitability.

Discussion

This longitudinal study of patients with progressive MS showed significant disability progression and cortical excitability changes over a one-year follow-up in untreated patients (group 2), but not in treated patients (group 1). The absence of clinical worsening in group 1 could be explained by the fact that even a two-year follow-up may be too short to measure disease progression in progressive MS (Ebers et al., 2008). However, the group 1 patients, who were younger and tended to have a shorter disease duration than those in group 2, should have evolved more significantly, since functional worsening is faster in the early phase of progressive MS. Although, so far, no consistent efficacy of immunomodulatory therapy has been demonstrated in progressive MS (Comi, 2013), the unexpected absence of disease progression in group 1 might be attributed to the efficacy of the treatment. Unfortunately, due to the small sample sizes, it was not possible to distinguish between the effects of the different drugs.

In clinical practice, MEP amplitude and latency and central motor conduction time (CMCT) are the TMS parameters most widely used to assess MS patients (Ravnborg et al., 1992; Fuhr et al., 2001; Kalkers et al., 2007; Kale et al., 2009; Bejarano et al., 2011), although CMCT, for instance, does not correlate with brain lesion load in progressive MS (Facchetti et al., 1997). We found that MEP amplitude negatively correlated with EDSS scores at baseline, but neither amplitude nor latency of MEPs changed over time; CMCT was not measured, which was a significant limitation of our study.

Actually, cortical excitability measurements appeared valuable to show disease progression, which was revealed by rMT increase and SICI decrease. On the one hand, rMT provides global information on the excitability and membrane properties of cortical pyramidal neurons, but this parameter is not independent of corticospinal output elements, downstream of the motor cortex (Ziemann et al., 1996; Chen et al.,

1997). On the other hand, SICI reflects the recruitment of intracortical GABAergic inhibitory pathways, but this parameter is also influenced by complex interactions with glutamatergic pathways in the motor cortex (Ziemann, 1999; Paulus et al., 2008). The SICI reduction found in the present study probably does not correspond to a change in the regulation of GABA/glutamate balance favoring facilitation mechanisms, as the rMT concomitantly increased. Actually, the SICI reduction more likely reveals an increased threshold of inhibitory controls in the altered motor cortex. This could be at the origin of adaptive neuronal plasticity, since inhibition plays a crucial role in limiting the plastic properties of cortical tissue (Baroncelli et al., 2011). A similar pattern of increased rMT and decreased SICI was previously found in relapsing MS patients (Caramia et al., 2004) and in other studies was seen to be more marked in patients with secondary progressive MS compared to relapsing-remitting patients or healthy controls (Conte et al., 2009; Vucic et al., 2012). In those studies, SICI reduction significantly correlated with EDSS increase. By contrast, Mori et al. (2013) found neither SICI difference between progressive and relapsing-remitting forms of MS nor a correlation between SICI and EDSS scores. In their study, which included a large majority of relapsing-remitting forms, only a reduction in short-interval ICF was found to correlate with disability score worsening. Short-interval ICF, measured at a short ISI (1.5 ms), is totally different from the type of ICF measured in the present study. Finally, SICI has been found to be reduced in MS patients with fatigue but not in MS patients without fatigue compared to healthy controls (Liepert et al., 2005).

In most of these previous studies (Caramia et al., 2004; Liepert et al., 2005; Conte et al., 2009; Mori et al., 2013), cortical excitability was assessed by focal stimulation of the primary motor cortex using a figure-of-eight coil. Conversely, as in Vucic et al. (2012) and in a previous study of MS patients published by our team (Ayache et al., 2014), in the present study a circular coil was preferred to a figure-of-eight coil in order to improve the precision of coil repositioning and the reliability of repeated TMS measurements of cortical excitability. By centering the circular coil on the vertex, cortical mapping was not necessary in order to locate the hotspot a few cm lateral to the vertex (a procedure that is, instead, necessary with a figure-of-eight coil). However, the use of a circular coil makes the stimulation less focal and the current generated within the cortex may spread beyond the primary motor area to the premotor and parietal regions. Between these regions, there are cortico-cortical connections that can be investigated by TMS (Koch and Rothwell, 2009) and the two motor generators (premotor and primary motor areas) show different behavior when a circular coil is centered over the vertex or lateralized (Baykushev et al., 2008). In any case, whatever the precise sensorimotor circuits explored, our results are relevant for assessing the effects of disease-modifying drugs on cortical excitability. In this

study, these effects were studied longitudinally for the first time in a cohort of patients with progressive MS. At baseline, EDSS scores correlated negatively with MEP amplitude and positively with rMT/aMT values. During follow-up, further rMT increase and SIC1 reduction were observed, although these were not correlated with EDSS worsening. This could be due to the sample being too small or the follow-up too short. EDSS also has a limited sensitivity to assess motor performance, being biased toward only one dimension of motor disability related to pyramidal dysfunction, i.e. walking capacity (Hoogervorst et al., 2001).

Caramia et al. (2004) hypothesized that processes of inflammation and acute demyelination observed in MS relapses lead to decreased neuronal excitability in the motor cortex. These changes in cortical excitability may be due to a deleterious influence of the local inflammatory environment, since it has been shown that various inflammatory cytokines, e.g. interleukins, can affect neuronal function and cortical excitability in MS (Mori et al., 2014). Both aspects can be improved concomitantly by immunomodulatory therapy in relapsing-remitting MS patients (Mori et al., 2012). According to the results reported by Conte et al. (2009) and Vucic et al. (2012), these changes could become permanent when the degenerative process, including neuronal loss and cortical atrophy, takes place in patients with progressive MS.

For now, EDSS score is the main outcome measure used in most clinical trials in patients with progressive MS. Cortical excitability parameters, such as rMT and SIC1, could be biomarkers sensitive to treatment-induced changes in MS patients, useful for documenting motor cortex alteration and disease progression or severity.

In a pilot study, we recently showed that a combined treatment by iron depletion induced by bloodletting followed by recombinant human erythropoietin (EPO) administration could improve fatigue and walking capacities in patients with progressive MS, in parallel to cortical excitability changes assessed by TMS (Créange et al., 2013). Among these changes, rMT was significantly decreased following chronic EPO administration.

In another study, we assessed the effects of high-dose steroids on cortical excitability in the treatment of acute MS relapse (Ayache et al., 2014). Steroids further reduced the SIC1, already low at baseline. This observation seems to contradict the present results. However, steroid effects were assessed immediately after three days of acute treatment and therefore cannot be compared with the effects of a chronic treatment, as in this study. We assume that acute steroid treatment transiently facilitates the transmission of information within the motor cortex (immediately reducing the SIC1), while chronic immunomodulatory therapy maintains the excitability of the motor cortex, including inhibitory circuits (preventing SIC1 decrease).

In conclusion, this first longitudinal study of cortical excitability parameters in patients with progressive

MS must be considered a pilot study, because of various limitations, such as the small sample size, the patients' heterogeneous clinical profile and disease duration, the use of different immunomodulatory drugs, and the absence of correlation with the presence of gadolinium-enhancing lesions or lesion location on brain magnetic resonance imaging (MRI). Therefore, further studies are required to confirm these preliminary results and the potential value of rMT and SIC1 measurements for follow-up in a larger population of patients with progressive MS.

It would also be interesting to evaluate TMS parameters of interhemispheric cortical excitability, which have been found to correlate with clinical and MRI measures of MS progression (Codecà et al., 2010). Finally, combined measures of multimodal evoked potentials, including MEPs, have been found to be reliable predictors of disability in MS at short-term and long-term assessments, both in primary progressive MS (Schlaeger et al., 2014a) and in relapsing-remitting MS, if performed in the relapse-free interval (Schlaeger et al., 2014b). The value of adding cortical excitability parameters to these combined measures of evoked potentials also deserves further investigation.

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