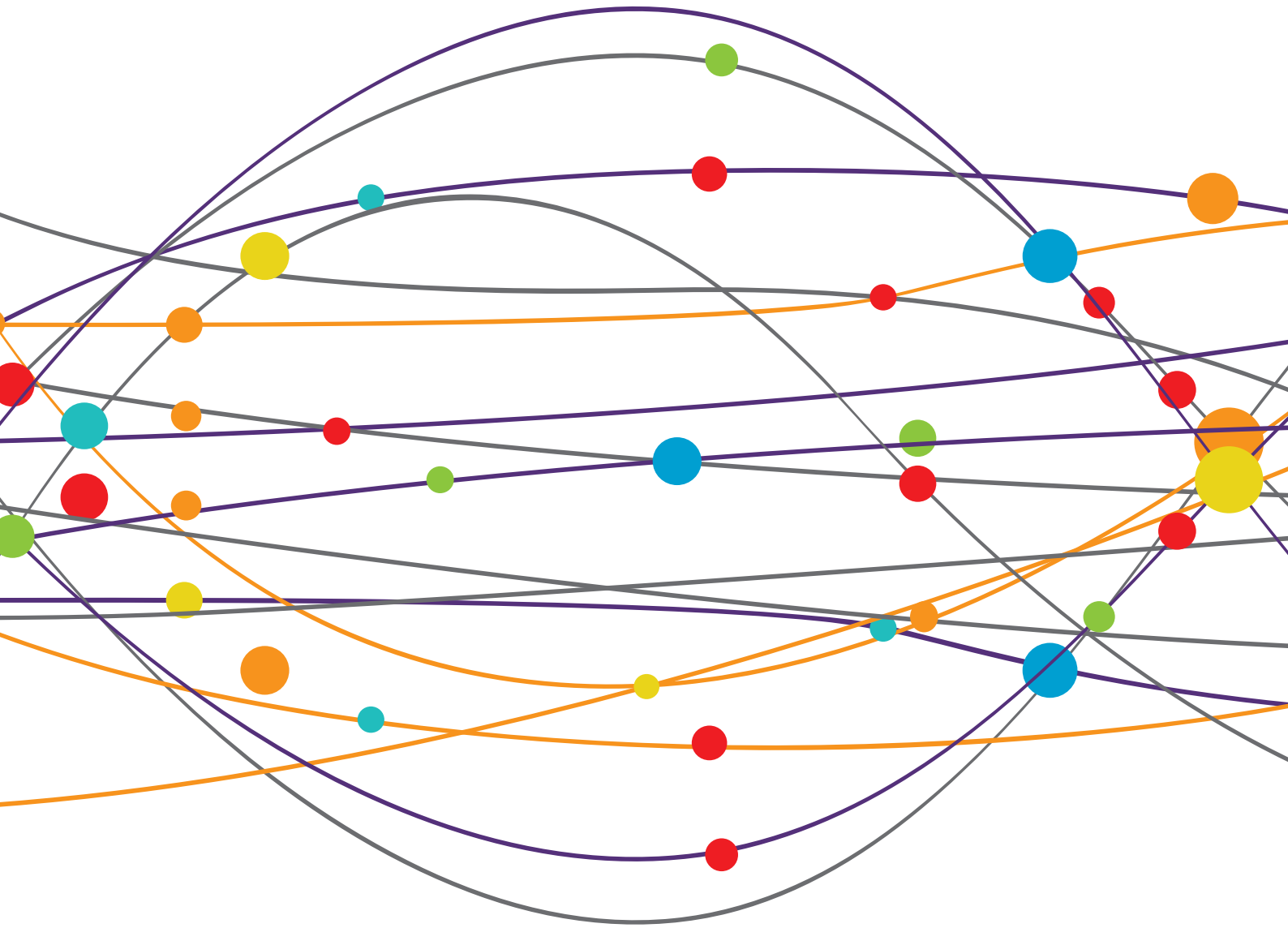
CORTICOSPINAL EXCITABILITY IN PATIENTS WITH MULTIPLE SCLEROSIS

EDITED BY: Samar S. Ayache, Ulrich Palm and Moussa Antoine Chalah
PUBLISHED IN: Frontiers in Neurology



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88966-490-0
DOI 10.3389/978-2-88966-490-0

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

CORTICOSPINAL EXCITABILITY IN PATIENTS WITH MULTIPLE SCLEROSIS

Topic Editors:
Samar S. Ayache, Hôpitaux Universitaires Henri Mondor, France
Ulrich Palm, Medical Park Chiemseeblick, Germany
Moussa Antoine Chalah, Hôpitaux Universitaires Henri Mondor, France

Citation: Ayache, S. S., Palm, U., Chalah, M. A., eds. (2021). Corticospinal Excitability in Patients with Multiple Sclerosis. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88966-490-0

Table of Contents

frontiers in Neurology

EDITORIAL
published: 12 January 2021
doi: 10.3389/fneur.2020.635612

Editorial: Corticospinal Excitability in Patients With Multiple Sclerosis

Moussa A. Chalah[1,2], *Ulrich Palm*[3,4] *and Samar S. Ayache*[1,2]*

[1] EA 4391, Excitabilité Nerveuse et Thérapeutique, Université Paris-Est-Créteil, Créteil, France, [2] Service de Physiologie - Explorations Fonctionnelles, Hôpital Henri Mondor, Assistance Publique - Hôpitaux de Paris, Créteil, France, [3] Department of Psychiatry and Psychotherapy, Klinikum der Universität München, Munich, Germany, [4] Medical Park Chiemseeblick, Bernau, Germany

Keywords: multiple sclerosis (MS), corticospinal excitability, TMS—transcranial magnetic stimulation, tDCS—transcranial direct current stimulation, non-invasive brain stimulation

Editorial on the Research Topic

Corticospinal Excitability in Patients With Multiple Sclerosis

A plethora of debilitating symptoms can be caused by multiple sclerosis (MS) such as sensory and motor dysfunction, cognitive impairment, mood disorder, and fatigue (1–3). Such complaints can drastically impact a patient's quality of life, are mostly assessed in a clinical manner or with the help of scales, questionnaires, or test batteries, and cannot be completely or partially relieved using pharmacotherapeutics. Moreover, their underlying mechanisms are not fully elucidated. Therefore, probing corticospinal excitability as a surrogate of the neuronal network function by using transcranial magnetic stimulation (TMS) could help in further understanding the underlying mechanisms of MS (4). TMS consists of applying a magnetic field over the scalp in a single- or double-pulse paradigm so as to obtain variables which reflect the functioning of the corticospinal system (4). These measures assess the excitability of the neuronal membrane [e.g., motor threshold (MT) and motor evoked potentials (MEP) amplitude], and the function of intracortical GABAergic and glutamatergic circuits [reflected by the intracortical inhibition (ICI) and facilitation parameters, respectively] as well as other processes [e.g., corticospinal inhibition (CSP) or interhemispheric transcallosal mechanisms; (4)]. This Research Topic focused on the exploration and modulation of corticospinal excitability in MS.

First, applying TMS could help in identifying biomarkers of the disease itself. In their perspective article, Bassi et al. reported an association between some underlying mechanisms of MS (demyelination and axonal loss) and TMS measures [e.g., low amplitudes and high latencies of MEP, high resting MT, and increased central motor conduction time (CMCT)]. In addition, an inflammation-mediated synaptopathy seems to underlie a hyperexcitability state which could appear, using TMS, as an imbalance between cortical excitatory and inhibitory processes. This could occur early in the disease process, seems to characterize relapses, and can become marked along the disease progression. Besides conventional TMS measures, new paradigms might give an additional scope on the neurophysiology of MS. For instance, by applying dual-site TMS of the ipsilateral dorsal premotor cortex (PMd) and primary motor cortex (M1), Ruiu et al. demonstrated in their original article a preserved PMd-M1 connectivity in patients with relapsing-remitting MS. Keeping this in mind, it would also be interesting to evaluate this outcome in patients with other disease phenotypes.

Second, TMS could also help in monitoring the disease evolution, especially when facing difficulties in documenting clinical progression. In their original article involving patients with progressive MS, Hardmeier et al. validated TMS-derived quantitative scores (i.e., CMCT and corticomuscular latency) that were obtained, along with clinical measures (disability and

OPEN ACCESS

Edited and reviewed by:
Robert Weissert,
University of Regensburg, Germany

***Correspondence:**
Samar S. Ayache
samarayache@gmail.com

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *30 November 2020*
Accepted: *08 December 2020*
Published: *12 January 2021*

Citation:
Chalah MA, Palm U and Ayache SS (2021) Editorial: Corticospinal Excitability in Patients With Multiple Sclerosis. Front. Neurol. 11:635612. doi: 10.3389/fneur.2020.635612

ambulation scores) over a 2-year period. Only neurophysiological measures were significantly deteriorated at 1 year, and higher effect size was obtained for neurophysiological worsening (mostly the mean corticomuscular latency) compared to clinical worsening at 2 years. These findings support the ability of neurophysiological measures to detect subtle changes before the appearance of clinically palpable progression, highlighting their potential add-on value to clinical assessment.

Third, besides the disease underlying mechanisms, MS symptoms could be explored using TMS, as reviewed by Bassi et al. For instance, anxiety was associated with interhemispheric inhibition in one study. In another study, verbal memory deficits were associated with defective short-latency afferent inhibition, a variable reflecting motor cortex cholinergic activity. In a few other works, fatigue was related to abnormal GABAergic inhibition (short-interval ICI and CSP). The latter finding was also highlighted in the review by Capone et al. who provided insight on the application of TMS, as well as other neurophysiological modalities [i.e., electroencephalography, electromyography (EMG), event related potentials, autonomic measures, and polysomnography], to explore fatigue. Capone et al. suggested the main contribution of central mechanisms to this symptom. In addition to the previous literature, Ruiu et al. described a correlation between fatigue scores and a decrease in functional connectivity during cued motor inhibition, suggesting the latter as a promising marker of MS fatigue.

Fourth, TMS measures could constitute potential outcomes for rehabilitation interventions. In the original article by Chaves et al. involving patients with progressive MS, 10 weeks of walking training resulted in significant enhancement of corticospinal excitability that was observed right after the intervention, but not 3 months later (active MT, MEP amplitude, and recruitment curve in both hemispheres; CSP in the hemisphere corresponding to the less affected hand). Some corticospinal excitability changes were significantly correlated with fatigue improvement.

Fifth, besides exploring corticospinal excitability, it is also possible to modulate it using non-invasive brain stimulation techniques such as TMS, as well as transcranial direct current stimulation (tDCS) (4, 5). Relative to TMS, tDCS is portable, easier to handle, and has a lower cost (5). As shown in the review by Capone et al., most of the available studies employed tDCS and focused on MS fatigue. Anodal tDCS was applied over cortical areas that take part in the MS fatigue loop [i.e., left dorsolateral prefrontal cortex (DLPFC), right posterior parietal cortex, and bilateral somatosensory and/or motor cortices], and predominantly yielded significant antifatigue effects. This highlights the potential utility of this technique in managing MS fatigue. In addition to fatigue, walking and functional mobility impairments could be targeted with tDCS. This was studied by Pilloni et al. who performed a randomized, sham-controlled, proof-of-concept study in which a 20-min session of anodal M1 tDCS coupled with aerobic exercise did not result in clinical improvement (gait speed and time). This lack of effects might be attributed to the number of sessions, as the authors recently reported significant effects when performing multiple sessions (6). A third domain to target would be cognitive impairment. In their mini-review, Nasios et al. reappraised the underlying mechanisms of cognitive deficits in MS (regional tissue damage and atrophy, synaptopathy, and cognitive network dysfunction) and emphasized the need for further research to assess the effects of neuromodulation in this context. In the original article by Grigorescu et al., five consecutive daily sessions of bifrontal tDCS applied in a randomized sham-controlled manner did not ameliorate general or social cognition (i.e., attention, information processing speed, working memory, or theory of mind). Furthermore, working memory improvement (1-back test accuracy) was only observed after sham intervention, which might reflect a potential impairment of working memory following bifrontal tDCS that could be attributed to the cathode placement over the right DLPFC.

Finally, besides central non-invasive brain stimulation techniques, other approaches could be of help in harnessing neuroplasticity processes. In their mini-review, Thompson and Sinkjær presented a training method, the operant conditioning of EMG-evoked responses, as a way to enhance corticospinal excitability and consequently the motor function in MS. Via an up-conditioning training of the corticospinal system behavior, the rewarded excitability state could be learned and retained in daily life by means of iterative training. Here, one should note the relevance of assessing inflammation and cognitive status when studying the effects of this learning technique, since it relies on cognitive functions that could be halted in MS and on synaptic plasticity that could be hampered by inflammation.

Taken together, the available data suggest the promising potential of exploring and modulating corticospinal excitability for research, diagnostic, and therapeutic purposes. The current limitations arise from the low number of studies, the small sample sizes, and the interstudy variations in methods or results. There is no doubt that this field is still in its early stages of development. Therefore, future large-scale works would help in overcoming the current challenges and providing further insights on the clinical utility of this approach. A greater understanding of the neurophysiological correlates of disease characteristics and symptoms could allow for designing of patient-tailored therapies. And, combining several therapies depending on the clinical context (e.g., brain stimulation, operant conditioning of EMG-evoked responses, cognitive rehabilitation, exercise training, psychotherapies, or medications) might result in synergistic effects on the studies' outcomes.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

REFERENCES

1. Zackowski KM, Wang JI, McGready J, Calabresi PA, Newsome SD. Quantitative sensory and motor measures detect change overtime and correlate with walking speed in individuals with multiple sclerosis. *Mult Scler Relat Disord*. (2015) 4:67–74. doi: 10.1016/j.msard.2014.11.001
2. Ayache SS, Chalah MA. Fatigue and affective manifestations in multiple sclerosis-a cluster approach. *Brain Sci*. (2019) 10:10. doi: 10.3390/brainsci10010010
3. Benedict RHB, Amato MP, DeLuca J, Geurts JJG. Cognitive impairment in multiple sclerosis: clinical management, MRI, and therapeutic avenues. *Lancet Neurol*. (2020) 19:860–71. doi: 10.1016/S1474-4422(20)30277-5
4. Lefaucheur JP, Aleman A, Baeken C, Benninger DH, Brunelin J, Di Lazzaro V, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014-2018). *Clin Neurophysiol*. (2020) 131:474–528. doi: 10.1016/j.clinph.2019.11.002
5. Lefaucheur JP, Antal A, Ayache SS, Benninger DH, Brunelin J, Cogiamanian F, et al. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). *Clin Neurophysiol*. (2017) 128:56–92. doi: 10.1016/j.clinph.2016.10.087
6. Pilloni G, Choi C, Shaw MT, Coghe G, Krupp L, Moffat M, et al. Walking in multiple sclerosis improves with tDCS: a randomized, double-blind, sham-controlled study. *Ann Clin Transl Neurol*. (2020) 7:2310–9. doi: 10.1002/acn3.51224

Conflict of Interest: MC declares having received compensation from Janssen Global Services LLC. UP has a private practice with NeuroCare Group, Munich, Germany. SA declares having received travel grants or compensation from Genzyme, Biogen, Novartis, and Roche.

frontiers in Neurology

MINI REVIEW
published: 06 March 2020
doi: 10.3389/fneur.2020.00147

Cognitive Impairment and Brain Reorganization in MS: Underlying Mechanisms and the Role of Neurorehabilitation

Grigorios Nasios[1]*, *Christos Bakirtzis*[2] and *Lambros Messinis*[3]

[1] Department of Speech and Language Therapy, School of Health Sciences, University of Ioannina, Ioannina, Greece, [2] Department of Neurology, The Multiple Sclerosis Center, AHEPA University Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece, [3] Neuropsychology Section, Departments of Neurology and Psychiatry, University of Patras Medical School, Patras, Greece

Multiple sclerosis (MS) is a chronic, immune-mediated, inflammatory, and degenerative disease of the central nervous system (CNS) that affects both white and gray matter. Various mechanisms throughout its course, mainly regarding gray matter lesions and brain atrophy, result in cognitive network dysfunction and can cause clinically significant cognitive impairment in roughly half the persons living with MS. Altered cognition is responsible for many negative aspects of patients' lives, independently of physical disability, such as higher unemployment and divorce rates, reduced social activities, and an overall decrease in quality of life. Despite its devastating impact it is not included in clinical ratings and decision making in the way it should be. It is interesting that only half the persons with MS exhibit cognitive dysfunction, as this implies that the other half remain cognitively intact. It appears that a dynamic balance between brain destruction and brain reorganization is taking place. This balance acts in favor of keeping brain systems functioning effectively, but this is not so in all cases, and the effect does not last forever. When these systems collapse, functional brain reorganization is not effective anymore, and clinically apparent impairments are evident. It is therefore important to reveal which factors could make provision for the subpopulation of patients in whom cognitive impairment occurs. Even if we manage to detect this subpopulation earlier, effective pharmaceutical treatments will still be lacking. Nevertheless, recent evidence shows that cognitive rehabilitation and neuromodulation, using non-invasive techniques such as transcranial magnetic or direct current stimulation, could be effective in cognitively impaired patients with MS. In this Mini Review, we discuss the mechanisms underlying cognitive impairment in MS. We also focus on mechanisms of reorganization of cognitive networks, which occur throughout the disease course. Finally, we review theoretical and practical issues of neurorehabilitation and neuromodulation for cognition in MS as well as factors that influence them and prevent them from being widely applied in clinical settings.

Keywords: multiple sclerosis, cognitive impairment, brain reorganization, cognitive rehabilitation, neuromodulation

OPEN ACCESS

Edited by:
Moussa Antoine Chalah, Hôpitaux Universitaires Henri Mondor, France

Reviewed by:
Rosa Iodice, University of Naples Federico II, Italy
Ralf Lürding, University of Regensburg, Germany

***Correspondence:**
Grigorios Nasios
grigoriosnasios@gmail.com

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *06 January 2020*
Accepted: *14 February 2020*
Published: *06 March 2020*

Citation:
Nasios G, Bakirtzis C and Messinis L (2020) Cognitive Impairment and Brain Reorganization in MS: Underlying Mechanisms and the Role of Neurorehabilitation. Front. Neurol. 11:147. doi: 10.3389/fneur.2020.00147

INTRODUCTION

People living with Multiple sclerosis (pwMS) commonly exhibit cognitive deficits, which negatively affects them multidimensionally (1). Daily functioning, decision making, vocational activities, marital status, socialization, behavior, mood, balance and mobility, and compliance with medications can be affected. The medical community, due to their often subtle nature and the difficulty that exists in detecting these deficits during routine clinical practice, was initially slow to appreciate them as a core clinical symptom of MS. We recently proposed a practical algorithm for clinicians regarding "what," "why," "how," and "when" to measure (2). Today, most of the evidence suggests that cognitive impairment in MS patients is present during all disease stages and across all disease clinical subtypes (3–5). CI can be detected even prior to diagnosis (6) in radiologically isolated syndrome (RIS) (7), clinically isolated syndrome (CIS) (8), "benign" MS (9), and the pediatric MS population (10). Deficits appear to be more frequent and widespread in the progressive rather than the relapsing form of the disease (11–13). Even then, roughly half of the patients do not exhibit prominent CI. CI is also linked to disease duration (12, 14), tissue damage and atrophy (15–17), and cognitive network efficiency (18, 19). Some cognitive domains appear to be more commonly compromised than others: information processing efficiency, episodic memory, attention, and executive functioning are found predominantly to be detrimentally affected in MS (20, 21). Among these domains the most common pattern involves circumscribed deficits as a combination of one or two of the abovementioned domains (e.g., attention/processing speed, learning/ memory, and or executive functions).

On the other hand, social cognitive deficits are an underestimated but important aspect of impairment in MS, reflecting how people process, store, and apply information in social interactions. Deficits in these domains have been associated with reduced quality of life, even after controlling for severity and duration of the disease, age, and neurocognitive performance (22, 23). This type of impairment is not entirely dependent on and parallel to general cognitive dysfunction—some patients experience disorganization in their social life before significant or detectable cognitive impairment is evident. The decrease in performance of social cognition (SC) tasks may reflect changes in brain activity and brain structure, either general or regional (22, 24).

In order to answer the question of why half of pwMS do not exhibit CI, approaching the disease within the context of its trilateral interference of tissue damage, tissue repair, and brain reorganization (25) may be helpful: tissue damage is indeed a matter of time and disease type and severity, and it can be partially influenced by the early introduction of efficacious disease modifying therapies (DMTs). Tissue repair is served by various mechanisms that are not yet well-illuminated, may vary in affected individuals, is altered by factors such as co-morbidity, stress, or lifestyle, and, unfortunately, was not targeted with specific medications until now; functional brain reorganization, in other words neuroplasticity, is the intrinsic force fighting the consequences of disease progression and can hopefully be managed through neurorehabilitation interventions.

In the following sections we have addressed issues concerning CI and functional brain reorganization in MS; we focused on cognitive network alterations, efficiency, and collapse, the role of inflammation, and mechanisms underlying synaptopathy and synaptogenesis. We further discussed the potential role of cognitive rehabilitation and neuromodulation in retaining and enhancing network efficiency in a clinically meaningful way.

Networks-Connectivity-Brain Reorganization

Brain reorganization in MS is studied intensively by mainly functional neuro-imaging methods. Functional connectivity (FC) at rest and during tasks can detect both hyperconnectivity and hypoconnectivity in brain networks. This can compensate for tissue damage, allowing pwMS to adequately cope with everyday cognitive tasks despite continuing structural brain damage. These alterations can be adaptive or maladaptive. We recently summarized the basic concepts, and limitations, of functional brain reorganization in MS (26). Even from early disease phases—in patients with CIS—dynamic changes in functional brain networks have been observed, resulting in the maintenance of normal efficiency in the brain and consequently representing a compensatory effect (27). A mixed pattern of hypoactivity and hyperactivity was found, by means of rs-fMRI, in pwMS at different stages of disease. Relapsing Remitting Multiple Sclerosis (RRMS) individuals with short disease duration, and RRMS with similar disabilities but longer disease duration, were characterized by a clearly distinct pattern of FC that involved predominantly sensory and cognitive networks, respectively (28). In a longitudinal 1-year network connectivity study, measures were compared between early RRMS patients and healthy matched controls as well as between patients with and without disease activity (29). The study reported that the strengthening of local network properties was only detectable in the cortex of patients and occurred independently of their disease activity. Authors discuss these changes as an adaptive mechanism that is important for maintaining brain function in response to neuroinflammation. In another study, patients who converted to MS exhibited significantly greater network connectivity at baseline than non-converters (30). Cader et al. concluded that both forms of adaptive functional change—that is, the enhancement of interactions between brain regions normally recruited, and the recruitment of alternative areas, or the use of complementary cognitive strategies—could limit clinical expression of the disease, particularly that of CI (31).

Abbreviations: CI, Cognitive impairment; CIS, clinically isolated syndrome; CNS, Central nervous system; CR, cognitive rehabilitation; DMTs, disease modifying therapies; FC, Functional connectivity; EAE, Experimental autoimmune encephalomyelitis; GABA, gamma-Aminobutyric acid; GM, Gray matter; LTD, long-term depression; LTP, long-term potentiation; MEP, Motor evoked potentials; MS, Multiple sclerosis; NIBS, Non-invasive brain stimulation; PPMS, Primary progressive MS; PwMS, people living with MS; RIS, radiologically isolated syndrome; RRMS, Relapsing-remitting MS; Rs-FC, resting-state Functional Connectivity; rs-fMRI, resting-state functional MRI; rTMS, repetitive transcranial magnetic stimulation; SC, Social cognition; SPMS, Secondary progressive MS; tDCS, transcranial direct current stimulation.

As a rule, PwMS perform better in cognitive tasks if they have preserved fMRI activity of their frontal lobes (32). Not only functional but also structural connectivity matters. Llufriu et al. investigating reorganization mechanisms at the structural level that are related to attention and executive performance in pwMS, and they found that the right pallidum and left insula within the frame of the brain's reorganization functioned as hubs in patients (33). However, we must keep in mind that several limitations exist that relate to the role of altered connectivity throughout the disease; it is still questionable whether the observed changes are relevant to cognitive performance and whether or not they are adaptive (26). As the disease progresses, network efficiency is challenged by tissue damage, restorative mechanisms become inadequate, and, finally, the network collapses (18).

Regional Tissue Damage and Atrophy

Gray matter (GM) lesions (15) and GM atrophy (16) play an important role in CI. Across the disease span, if left untreated, the white matter atrophy rate remains rather stable at 3-fold normal, but GM atrophy rate dramatically increases from 3.4-fold normal in CIS to 14-fold normal in SPMS (17). This localized GM atrophy has recently been found to be regionally selective, mainly involving deep structures, such as the thalamus, putamen, and caudate, and cortical regions, such as the sensorimotor cortex, insula, superior temporal, and cingulate gyrus, while these regions were functionally connected (34). In a 5-years follow-up study, it was shown that structural damage and especially cortical atrophy may predict cognitive decline in PwMS (35). Among strategic GM structures, the thalamus, basal ganglia, and hippocampus seem to play a central role. Thalamic volume declines faster in pwMS throughout the disease, and it was proposed to serve as a biomarker of degeneration (36). Its volume, shape, and function are related to cognitive performance in MS (37–39). In early RRMS patients (duration of disease <3 years), CI was detected in 28% over a 2-years follow up period, and in this subgroup a significant reduction in the percentage of thalamus volume was observed compared with the cognitively intact group (40). In a large cohort of MS patients, with various forms and stages of the disease, Rocca et al. investigated rs-FC abnormalities within the principal brain networks in PwMS (41). They found a complex pattern of decreased and increased rs-FC at a regional level: reduced thalamic rs-FC correlated with better neuropsychological performance, whereas, for all the remaining networks, reduced FC correlated with more severe clinical/cognitive impairment. This finding was in line with the observation of Zhou et al. who found that increased thalamic intrinsic oscillation amplitude in RRMS patients was associated with slowed cognitive processing, representing ineffective reorganization (42). Resting-state magneto-encephalography recordings from pwMS and healthy controls offered similar evidence, illustrating "the relationship between thalamic atrophy, altered functional connectivity and clinical and cognitive dysfunction in MS" (43). The importance of thalamic involvement in disease progression and CI is highlighted by Minagar et al. (44), who recommended that thalamic volume should be utilized as a biomarker in MS clinical trials. In a recent study where neuropsychological and MRI data of 375 PwMS were analyzed, altered performance on neuropsychological tests assessing attention and executive function was associated with caudate volume and posterior cingulate/precuneus atrophy, while tests primarily evaluating memory strongly correlated with thalamic volume (45). In untreated CIS patients, load-dependent dysfunction of the putamen was related to impaired performance during attention tasks (46). Amygdala atrophy was found to be the main predictor of impairment of social cognition (SC) in PwMS (24). In agreement with this, Pitteri at al. correlated bilateral amygdala damage, as measured by cortical lesion volume (CLV), to affected SC in PwMS, even in the absence of CI (47). In a multicenter study of structural correlates of CI in MS, the best predictors of CI were found to be atrophy of the hippocampus and deep GM nuclei (48). The importance of structural and functional integrity of the hippocampus was highlighted by Sumowski et al. (49), who investigated the neural basis of reserve against memory decline in PwMS, linking greater intellectual enrichment and better memory to larger hippocampal volume and supporting the argument that larger hippocampal volume is a key component of reserve against memory decline in MS. The hippocampus of PwMS usually has a high lesion load, demyelination, neuronal damage, synaptic dysfunction, neurotransmitter level reduction, and disconnection, linking hippocampus pathology not only to CI but also to the reorganization capacity of broader networks (48, 50–55). A biomarker indicating deep GM structures, especially thalamus and hippocampus volume and status, could ideally give provision of the cognitive status, insights for reorganization dynamics, and cues for therapeutic decisions.

Synaptopathy in MS

We formerly recognize MS as a myelin-targeting autoimmune disease of the CNS, causing inflammation, white (and gray) matter tissue damage, and neurodegeneration, but the influence of GM pathology was recognized later. Loss and malfunction of synapses could offer an explanation for this role. Recent clinical and experimental studies link inflammation to neurodegeneration, illuminating the contribution not only of visible structural damage but also of synaptic dysfunction in the pathophysiology of both motor and cognitive functions in MS. Many studies have provided robust evidence for diffuse synaptic dysfunction being present in both MS and EAE (experimental autoimmune encephalomyelitis, the animal model of MS) throughout the disease course (56–59). Stampanoni Bassi et al. and Mandolesi et al. have discussed thoroughly the molecular and cellular mechanisms underlying alterations in synaptic function and structure (58, 59). They emphasized the role of inflammation in neurotransmitters' imbalance; increased glutamate-mediated and reduced GABA-mediated signaling along with the excitotoxic effects of increased glutamate levels in the synaptic cleft may lead to synaptic degeneration, which, interestingly, may occur independently of GM demyelination and neuronal loss. Of course, synaptopathy can also be the consequence of axonal damage, but it is present from the initial phase of the disease when one could not yet expect that much of axonal damage. This supports the idea that synaptopathy, rather than axonal loss, leads to accumulation of disability, at least early

in the disease course. Focusing our attention to the synaptic level, we must keep in mind the role of long-term potentiation (LTP) and long-term depression (LTD) which represent core underlying mechanisms of synaptic plasticity, i.e., maintaining synaptic strengths, efficacy, and stability, adjusted dynamically by neural activity. The ways structural and functional damage result in synaptic failure, network dysfunction, and, therefore, CI have recently been reviewed by Di Filippo et al. (60).

Fortunately, and unlike the loss of neurons, the loss of synapses is reversible. New synapses can be generated, and dysfunctional synapses can be repaired, resulting in restoration of functions or even reversing the progression of the disease, as has been shown both in EAE animals (61) and in pwMS (62). Targeting synapses therapeutically can be achieved by at least some of the DMTs, especially those that pass the blood–brain barrier, by reducing inflammation and tissue damage or even by exerting direct neuroprotective effects (59). Since MS-related disability progression can be modulated by plasticity, and plasticity can be enhanced by neurorehabilitation and neuromodulation, these latter approaches could be therapeutically used to delay progression; this promotes brain reorganization, mainly at the synaptic level, since their mechanisms of action include LTP/LTD.

Neurorehabilitation and Neuromodulation for MS-Related Cognitive Impairment

Three decades have passed since the first published reports under the search items "cognitive rehabilitation" and "MS" appeared in Pubmed (1,086 research items in total, two in year 1990, 160 in 2019, page visited on 1.1.2020). In 1993, DeLuca and Johnson stated that, since cognitive dysfunction negatively impacts the lives of pwMS, it must be targeted by neurorehabilitation (63). They described the complicated landscape of cognitive rehabilitation (CR) in MS since, due to "the heterogeneous nature of the CNS lesions, each person with MS brings a unique pattern of cognitive difficulties," and, furthermore, "effective CR in MS goes beyond simple assessment and treatment of specific deficits" (63). Since then, more questions than answers have arisen. Questions regarding the evaluation of CI and the type of CR should be investigated (64); evidence and methodological restrictions of CR protocols (1, 65–68) as well as many practical issues of CR, such as the mechanisms of action, duration, intensity, frequency, repeatability, consistency and duration of effects, ecological validity, and "the transportability of such interventions under real-world conditions," (69) should be fully explored. There is another major practical restriction: in most countries, there is lack of providers (clinical neuropsychologists and trained speech language therapists) able to apply these methods (64). These restrictions are reflected in the low rate of pwMS exposed to CI, even in countries, such as Finland, with high incidences of MS and advanced health services (70); pharmacological treatments for MS-related CI are still lacking (64, 69, 71). In order to move faster from the research fields to clinical grounds and offer CR as standard-of-care treatment, a roadmap was recently proposed by Sandroff and DeLuca (69). One major point concerning CR for MS-related CI is its mechanism of action, which seems to be the enhancement of neuroplasticity. Prosperini et al. reviewed the literature, showing that both motor and cognitive rehabilitation enhance functional and structural brain plasticity in pwMS, and this enhancement is specifically linked to the trained domain (72). Recently, Prosperini and Di Filippo updated evidence from animal models and pwMS on plasticity following rehabilitation (73).

Neuromodulation is technology acting directly upon the nervous system. Non-invasive brain stimulation (NIBS) refers to the application on the scalp of a changing magnetic field [transcranial magnetic stimulation, TMS; (74)], or low-intensity electrical current [transcranial direct current stimulation, tDCS; (75)] over a short period of time, both of which are methods capable of altering brain function since they have cumulative and long lasting effects. The reader is referred to relevant, recently-published reviews for the use of rTMS (26) and tDCS (76) in the management of MS-related symptoms. Both techniques are easily applicable, affordable, and rather safe, with tDCS being much cheaper, able to be self-administered at home by remote supervision (77) and, more importantly, while performing a task ("on-line"), while rTMS must be carried out in the presence of a skilled clinician and during rest ("off-line"). Changing stimulus parameters and/or electrode polarity, excitation (high-frequency rTMS or anodal tDCS), or inhibition protocols (low-frequency rTMS or cathodal tDCS) can be designed, inducing LTP-like and LTD-like plasticity, respectively; this also influences brain plastic changes, acting therapeutically, either alone or in combination with CR and/or exercise. The neurobiological basis and the effectiveness of NIBS for MS-related symptoms have been updated by Leocani et al. (78). Among the issues are the differences from patient to patient in the electrical current flow induced by NIBS techniques; these depend on the volume and topography of the lesions, and different patients may need different NIBS protocols. However, since the use of tDCS during cognitive rehabilitation may improve outcomes and provide beneficial results in a short time (79), newer large-scale studies should further be performed in order to provide robust evidence to support the implementation of NIBS in routine practice (80).

DISCUSSION

Here, we have discussed the mechanisms underlying cognitive impairment and the reorganization of cognitive networks in MS and issues for the implementation of neurorehabilitation and neuromodulation in clinical settings. Recently, Harel et al. presented "the bright side" of cognitive function in multiple sclerosis (81). Indeed, nearly 20 years after the disease onset, more than three out of 10 the pwMS of their large sample were cognitively intact. But there is also a "dark side": two out of 10 were seriously cognitively handicapped, while one of 10 were both severely affected cognitively and physically. Furthermore, the disease does not last only 20 years, but it is lifelong; the mean age of pwMS in this study was noted as 49.3 years, meaning that the majority of them will still be alive 10–20 years later, the proportion of disabled will definitely increase, as will co-morbidities (82), and their treatment opportunities

will be narrowed. Unfortunately, as it has been shown in the EAE model of MS, the loss of synapses can occur early in the disease course, irrespectively of demeylination (83). Also, neurodegeneration and resulted atrophy is proven to be evident, subclinically, even from the radiologically isolated syndrome (84). These two mechanisms could explain why CI can be present even before the time of diagnosis. Additionally, what we have undoubtedly learned is that CI at diagnosis predicts worse future disease progression (85), impairment of specific cognitive sub-domains might better predict progression (86), and patients with pediatric onset MS are more likely to have CI than patients with disease onset in adulthood, independent of age, or disease duration (87). A patient with less severe tissue damage, less atrophy, spared key brain loci, more effective tissue repair mechanisms, and enhanced brain reorganization capacities could remain cognitively intact, even decades after disease onset, and vice versa. In the first instance, the patient will probably constitute "the bright side" of the ∼50% cognitively intact pwMS. What about the other 50%? For them, more than the others, "time is brain," and delays in appropriate clinical decisions will probably cost their transition to the "dark side" of CI. In our opinion, early clinical detection of CI, coupled with evidence of structural damage in key brain regions (thalamus, hippocampus, amygdala, etc.) and altered network connectivity, could serve toward this goal. Therefore, there is an urgent need to identify this high-risk subpopulation of pwMS, and, since it is not possible to be achieved early in the disease course through clinical and conventional neuroimaging grounds only (88), we have to find biomarkers (molecular, metabolic, imaging, and clinical) to detect earlier CNS pathology "before structural tissue damage has become definite" (89). Impaired cognition is associated with early increases in FC, which then decreases due to the exhaustion of compensating mechanisms, forming the "inverted U" rs-FC curve (89). Indeed, patients who converted to MS exhibited "significantly greater network connectivity at baseline than non-converters" and a "subsequent connectivity loss over time, not observed in the non-converters' network" (30, 90). Therefore, despite methodological difficulties (91), widely available imaging markers could soon offer more (92). As we have previously stated, a biomarker composed of deep GM structures, and especially the thalamus and hippocampus volume and status, could "reflect" the cognitive status and provide insight into reorganization dynamics. Once recognized, this subpopulation should be treated more aggressively with highly efficacious DMTs and, ideally, neurorehabilitation and neuromodulation procedures.

We are not optimistic about the introduction of CR and NBIS in routine clinical care, at least in the near future, and there are reasons for this: neurorehabilitation is ultimately "treatment of the whole person" (69) and should, in other words, be tailored to every individual person. This means that it is almost impossible to include parameters of every pwMS—as they all have distinct disease characteristics, personalities, and life variables—into clinical trials and then translate their results back to a highly individualized procedure, maximizing the possibilities to identify the right patient and carry out the appropriate treatment. Moreover, as we discussed, there are many unsolved practical issues for their implementation in clinical practice. For NIBS techniques, additional issues arise, including identifying sites for brain stimulation, depending on brain lesion topography of every single pwMS, and simultaneously choosing excitatory or inhibitory protocols, or combination of both. These sophisticated individualized treatments must be carried out by a large number of trained clinicians, who do not yet exist. Finally, the (large) financial cost must be covered somehow.

What is feasible? Diagnose MS sooner, detect CI earlier and include it in clinical decisions, start treatment early and constantly follow through with more effective DMTs, find medications for CNS tissue repair, adopt strategies for "reserve and brain maintenance" (93), and, of course, do more research on neurorehabilitation and neuromodulation since they seem to be at least in part effective, even in advanced disease stages (94), and may enhance the brain's plasticity and alter disease course. This is all with the ultimate goal of implementing these methods in routine MS management.

AUTHOR CONTRIBUTIONS

GN conceptualized this mini-review article. GN, CB, and LM wrote the first draft of the manuscript. All authors were involved in the critical reading and the revision process.

REFERENCES

1. Messinis L, Papathanasopoulos P, Kosmidis MH, Nasios G, Kambanaros M. Neuropsychological features of multiple sclerosis: impact and rehabilitation. *Behav Neurol*. (2018) 2018:4831647. doi: 10.1155/2018/4831647
2. Bakirtzis C, Ioannidis P, Messinis L, Nasios G, Konstantinopoulou E, Papathanasopoulos P, et al. The rationale for monitoring cognitive function in multiple sclerosis: practical issues for clinicians. *Open Neurol J*. (2018) 12:31–40. doi: 10.2174/1874205X01812010031
3. Prakash RS, Snook EM, Lewis JM, Motl RW, Kramer AF. Cognitive impairments in relapsing remitting multiple sclerosis: a meta analysis. *Multiple Sclerosis*. (2008) 14:1250–61. doi: 10.1177/1352458508095004
4. Langdon DW. Cognition in multiple sclerosis. *Curr Opin Neurol.* (2011) 24:244–9. doi: 10.1097/WCO.0b013e328346a43b
5. Rosti-Otajarvi E, Ruutianen J, Huhtala H, Hamalainen P. Cognitive performance profile in different phenotypes of MS with cognitive complaints. *Multiple Sclerosis Related Disord*. (2014) 3:463–72. doi: 10.1016/j.msard.2014.01.003
6. Cortese M, Riise T, Bjørnevik K, Bhan A, Farbu E, Grytten N, et al. Preclinical disease activity in multiple sclerosis: a prospective study of cognitive performance prior to first symptom. *Ann Neurol*. (2016) 80:616–24. doi: 10.1002/ana.24769
7. Amato MP, Hakiki B, Goretti B, Rossi F, Stromillo ML, Giorgio A, et al. Association of MRI metrics and cognitive impairment in radiologically isolated syndromes. *Neurology*. (2012) 78:309–14. doi: 10.1212/WNL.0b013e31824528c9
8. Zipoli V, Goretti B, Hakiki B, Siracusa G, Sorbi S, Portaccio E, et al. Cognitive impairment predicts conversion to multiple sclerosis in clinically isolated syndromes. *Mult Scler*. (2010) 16:62–7. doi: 10.1177/1352458509350311
9. Rovaris M, Riccitelli G, Judica E, Possa F, Caputo D, Ghezzi A, et al. Cognitive impairment and structural brain damage in benign multiple sclerosis. *Neurology*. (2008) 71:1521–6. doi: 10.1212/01.wnl.0000319694.14251.95

10. Charvet LE, O'Donnell EH, Belman AL, Chitnis T, Ness JM, Parrish J, et al. Longitudinal evaluation of cognitive functioning in pediatric multiple sclerosis: report from the US Pediatric Multiple Sclerosis Network. *Mult Scler*. (2014) 20:1502–10. doi: 10.1177/1352458514527862
11. Papathanasiou A, Messinis L, Georgiou LV, Papathanasopoulos P. Cognitive impairment in relapsing remitting and secondary progressive multiple sclerosis patients: efficacy of a computerized cognitive screening battery. *ISRN Neurology*. (2014) 2014:151379. doi: 10.1155/2014/151379
12. Potagas C, Giogkaraki E, Koutsis G, Mandellos D, Tsirempolou E, Sfagos C, et al. Cognitive impairment in different MS subtypes and clinically isolated syndromes. *J Neurol Sci*. (2008) 267:100–6. doi: 10.1016/j.jns.2007.10.002
13. Ntoskou A, Messinis L, Nasios G, Martzoukou M, Makris G, Panagiotopoulos E, et al. Cognitive and language deficits in multiple sclerosis: comparison of relapsing remitting and secondary progressive subtypes. *Open Neurol J*. (2018) 12:19–30. doi: 10.2174/1874205X01812010019
14. Achiron A, Chapman J, Magalashvili D, Dolev M, Lavie M, Bercovich E, et al. Modeling of cognitive impairment by disease duration in multiple sclerosis: a cross-sectional study (2013). *PLoS ONE*. 8:e71058. doi: 10.1371/journal.pone.0071058
15. Calabrese M, Favaretto A, Martini V, Gallo P. Grey matter lesions in MS: from histology to clinical implications. *Prion*. (2013) 7:20–7. doi: 10.4161/pri.22580
16. Calabrese M, Agosta F, Rinaldi F, Mattisi I, Grossi P, Favaretto A, et al. Cortical lesions and atrophy associated with cognitive impairment in relapsing-remitting multiple sclerosis. *Arch Neurol*. (2009) 66:1144–50. doi: 10.1001/archneurol.2009.174
17. Fisher E1, Lee JC, Nakamura K, Rudick RA. Gray matter atrophy in multiple sclerosis: a longitudinal study. *Ann Neurol*. (2008) 64:255–65. doi: 10.1002/ana.21436
18. Schoonheim MM, Meijer KA, Geurts JJ. Network collapse and cognitive impairment in multiple sclerosis. *Front Neurol*. (2015) 6:82. doi: 10.3389/fneur.2015.00082
19. Louapre C, Perlbarg V, García-Lorenzo D, Urbanski M, Benali H, Assouad R, et al. Brain networks disconnection in early multiple sclerosis cognitive deficits: an anatomofunctional study. *Hum Brain Mapp*. (2014) 35:4706–17. doi: 10.1002/hbm.22505
20. Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. *Lancet Neurol*. (2008) 7:1139–51. doi: 10.1016/S1474-4422(08)70259-X
21. Guimares J, Jose SA. Cognitive dysfunction in multiple sclerosis. *Front Neurol*. (2012) 3:74. doi: 10.3389/fneur.2012.00074
22. Chalah MA, Ayache SS. Deficits in social cognition: an unveiled signature of multiple sclerosis. *J Intern Neuropsychol Soc*. (2017) 23:266–86. doi: 10.1017/S1355617716001156
23. Giazkoulidou A, Messinis L, Nasios G. Cognitive functions and social cognition in multiple sclerosis: an overview. *Hell J Nucl Med*. (2019) 22(Suppl.102–10).
24. Batista S, d'Almeida OC, Afonso A, Freitas S, Macário C, Sousa L, et al. Impairment of social cognition in multiple sclerosis: Amygdala atrophy is the main predictor. *Mult Scler*. (2017) 1358–66. doi: 10.1177/1352458516680750
25. Rocca MA, De Meo E, Filippi M. Functional MRI in investigating cognitive impairment in multiple sclerosis. *Acta Neurol Scand*. (2016) 134(Suppl.200):39–46. doi: 10.1111/ane.12654
26. Nasios G, Messinis L, Dardiotis E, Papathanasopoulos P. Repetitive transcranial magnetic stimulation, cognition, and multiple sclerosis: an overview. *Behav Neurol*. (2018) 2018:8584653. doi: 10.1155/2018/8584653
27. Koubiyr I, Deloire M, Besson P, Coupé P, Dulau C, Pelletier J, et al. Longitudinal study of functional brain network reorganization in clinically isolated syndrome. *Mult Scler*. (2018) 27:1352458518813108. doi: 10.1177/1352458518813108
28. Castellazzi G, Debernard L, Melzer TR, Dalrymple-Alford JC, D'Angelo E, Miller DH, et al. Functional connectivity alterations reveal complex mechanisms based on clinical and radiological status in mild relapsing remitting multiple sclerosis. *Front Neurol*. (2018) 9:690. doi: 10.3389/fneur.2018.00690
29. Fleischer V, Koirala N, Droby A, Gracien RM, Deichmann R, Ziemann U, et al. Longitudinal cortical network reorganization in early relapsing-remitting multiple sclerosis. *Ther Adv Neurol Disord*. (2019) 12:1756286419838673. doi: 10.1177/1756286419838673
30. Tur C, Eshaghi A, Altmann DR, Jenkins TM, Prados F, Grussu F, et al. Structural cortical network reorganization associated with early conversion to multiple sclerosis. *Sci Rep*. (2018) 8:10715. doi: 10.1038/s41598-018-29017-1
31. Cader S, Cifelli A, Abu-Omar Y, Palace J, Matthews PM. Reduced brain functional reserve and altered functional connectivity in patients with multiple sclerosis. *Brain*. (2006) 129:527–37. doi: 10.1093/brain/awh670
32. Rocca MA, Valsasina P, Hulst HE, Abdel-Aziz K, Enzinger C, Gallo A, et al. Functional correlates of cognitive dysfunction in multiple sclerosis: a multicenter fMRI Study. *Hum Brain Mapp*. (2014) 35:5799–814. doi: 10.1002/hbm.22586
33. Llufriu S, Martinez-Heras E, Solana E, Sola-Valls N, Sepulveda M, Blanco Y, et al. Structural networks involved in attention and executive functions in multiple sclerosis. *Neuroimage Clin*. (2016) 13:288–96. doi: 10.1016/j.nicl.2016.11.026
34. Chiang FL, Wang Q, Yu FF, Romero RS, Huang SY, Fox PM, et al. Localised grey matter atrophy in multiple sclerosis is network-based: a coordinate-based meta-analysis. *Clin Radiol*. (2019) 74:816.e19–28. doi: 10.1016/j.crad.2019.07.005
35. Eijlers AJC, van Geest Q, Dekker I, Steenwijk MD, Meijer KA, Hulst HE, et al. Predicting cognitive decline in multiple sclerosis: a 5-year follow-up study. *Brain*. (2018) 141:2605–18. doi: 10.1093/brain/awy202
36. Azevedo CJ, Cen SY, Khadka S, Liu S, Kornak J, Shi Y, et al. Thalamic atrophy in multiple sclerosis: a magnetic resonance imaging marker of neurodegeneration throughout disease. *Ann Neurol*. (2018) 83:223–34. doi: 10.1002/ana.25150
37. Bisecco A, Capuano R, Caiazzo G, d'Ambrosio A, Docimo R, Cirillo M, et al. Regional changes in thalamic shape and volume are related to cognitive performance in multiple sclerosis. *Mult Scler*. (2019) 3:1352458519892552. doi: 10.1177/1352458519892552
38. Bergsland N, Zivadinov R, Dwyer MG, Weinstock-Guttman B, Benedict RH. Localized atrophy of the thalamus and slowed cognitive processing speed in MS patients. *Mult Scler*. (2016) 22:1327–36. doi: 10.1177/1352458515616204
39. Schoonheim MM, Hulst HE, Brandt RB, Strik M, Wink AM, Uitdehaag BM, et al. Thalamus structure and function determine severity of cognitive impairment in multiple sclerosis. *Neurology*. (2015) 84:776–83. doi: 10.1212/WNL.0000000000001285
40. Rojas JI, Murphy G, Sanchez F, Patrucco L, Fernandez MC, Miguez J, et al. Thalamus volume change and cognitive impairment in early relapsing-remitting multiple sclerosis patients. *Neuroradiol J*. (2018) 31:350–5. doi: 10.1177/1971400918781977
41. Rocca MA, Valsasina P, Leavitt VM, Rodegher M, Radaelli M, Riccitelli GC, et al. Functional network connectivity abnormalities in multiple sclerosis: correlations with disability and cognitive impairment. *Mult Scler*. (2018) 24:459–71. doi: 10.1177/1352458517699875
42. Zhou F, Zhuang Y, Wu L, Zhang N, Zeng X, Gong H, et al. Increased thalamic intrinsic oscillation amplitude in relapsing-remitting multiple sclerosis associated with the slowed cognitive processing. *Clin Imaging*. (2014) 38:605–10. doi: 10.1016/j.clinimag.2014.05.006
43. Tewarie P, Schoonheim MM, Stam CJ, van der Meer ML, van Dijk BW, Barkhof F, et al. Cognitive and clinical dysfunction, altered MEG resting-state networks and thalamic atrophy in multiple sclerosis. *PLoS ONE*. (2013) 8:e69318. doi: 10.1371/journal.pone.0069318
44. Minagar A, Barnett MH, Benedict RH, Pelletier D, Pirko I, Sahraian MA, et al. The thalamus and multiple sclerosis: modern views on pathologic, imaging, and clinical aspects. *Neurology*. (2013) 80:210–9. doi: 10.1212/WNL.0b013e31827b910b
45. Matías-Guiu JA, Cortés-Martínez A, Montero P, Pytel V, Moreno-Ramos T, Jorquera M, et al. Identification of cortical and subcortical correlates of cognitive performance in multiple sclerosis using voxel-based morphometry. *Front Neurol*. (2018) 9:920. doi: 10.3389/fneur.2018.00920
46. Tortorella C, Romano R, Direnzo V, Taurisano P, Zoccolella S, Iaffaldano P, et al. Load-dependent dysfunction of the putamen during attentional processing in patients with clinically isolated syndrome suggestive of multiple sclerosis. *Mult Scler*. (2013) 19:1153–60. doi: 10.1177/1352458512473671
47. Pitteri M, Genova H, Lengenfelder J, DeLuca J, Ziccardi S, Rossi V, et al. Social cognition deficits and the role of amygdala in relapsing remitting multiple

sclerosis patients without cognitive impairment. *Mult Scler Relat Disord*. (2019) 29:118–23. doi: 10.1016/j.msard.2019.01.030
48. Damjanovic D, Valsasina P, Rocca MA, Stromillo ML, Gallo A, Enzinger C, et al. Hippocampal and deep gray matter nuclei atrophy is relevant for explaining cognitive impairment in MS: a multicenter study. *AJNR Am J Neuroradiol*. (2017) 38:18–24. doi: 10.3174/ajnr.A4952
49. Sumowski JF, Rocca MA, Leavitt VM, Riccitelli G, Sandry J, DeLuca J, et al. Searching for the neural basis of reserve against memory decline: intellectual enrichment linked to larger hippocampal volume in multiple sclerosis. *Eur J Neurol*. (2016) 23:39–44. doi: 10.1111/ene.12662
50. van Geest Q, Hulst HE, Meijer KA, Hoyng L, Geurts JJG, Douw L. The importance of hippocampal dynamic connectivity in explaining memory function in multiple sclerosis. *Brain Behav*. (2018) 8:e00954. doi: 10.1002/brb3.954
51. Muhlert N, Atzori M, De Vita E, Thomas DL, Samson RS, Wheeler-Kingshott CA, et al. Memory in multiple sclerosis is linked to glutamate concentration in grey matter regions. *J Neurol Neurosurg Psychiatry*. (2014) 85:833–9. doi: 10.1136/jnnp-2013-306662
52. Sicotte NL, Kern KC, Giesser BS, Arshanapalli A, Schultz A, Montag M, et al. Regional hippocampal atrophy in multiple sclerosis. *Brain*. (2008) 131:1134–41. doi: 10.1093/brain/awn030
53. Geurts JJ, Bö L, Roosendaal SD, Hazes T, Daniëls R, Barkhof F, et al. Extensive hippocampal demyelination in multiple sclerosis. *J Neuropathol Exp Neurol*. (2007) 66:819–27. doi: 10.1097/nen.0b013e3181461f54
54. Rocca MA, Barkhof F, De Luca J, Frisén J, Geurts JJ, Hulst HE, et al. The hippocampus in multiple sclerosis. *Lancet Neurol*. (2018) 17:918–26. doi: 10.1016/S1474-4422(18)30309-0
55. Karavasilis E, Christidi F, Velonakis G, Tzanetakos D, Zalonis I, Potagas C, et al. Hippocampal structural and functional integrity in multiple sclerosis patients with or without memory impairment: a multimodal neuroimaging study. *Brain Imaging Behav*. (2019) 13:1049–59. doi: 10.1007/s11682-018-9924-y
56. Centonze D, Muzio L, Rossi S, Cavasinni F, De Chiara V, Bergami A, et al. Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. *J Neurosci*. (2009) 29:3442–52. doi: 10.1523/JNEUROSCI.5804-08.2009
57. Di Filippo M, de Iure A, Durante V, Gaetani L, Mancini A, Sarchielli P, et al. Synaptic plasticity and experimental autoimmune encephalomyelitis: implications for multiple sclerosis. *Brain Res*. (2015) 1621:205–13. doi: 10.1016/j.brainres.2014.12.004
58. Stampanoni Bassi M, Mori F, Buttari F, Marfia GA, Sancesario A, Centonze D, et al. Neurophysiology of synaptic functioning in multiple sclerosis. *Clin Neurophysiol*. (2017) 128:1148–57. doi: 10.1016/j.clinph.2017.04.006
59. Mandolesi G, Gentile A, Musella A, Fresegna D, De Vito F, Bullitta S, et al. Synaptopathy connects inflammation and neurodegeneration in multiple sclerosis. *Nat Rev Neurol*. (2015) 11:711–24. doi: 10.1038/nrneurol.2015.222
60. Di Filippo M, Portaccio E, Mancini A, Calabresi P. Multiple sclerosis and cognition: synaptic failure and network dysfunction. *Nat Rev Neurosci*. (2018) 19:599–609. doi: 10.1038/s41583-018-0053-9
61. Kerschensteiner M, Bareyre FM, Buddeberg BS, Merkler D, Stadelmann C, Brück W, et al. Remodeling of axonal connections contributes to recovery in an animal model of multiple sclerosis. *J Exp Med*. (2004) 200:1027–38. doi: 10.1084/jem.20040452
62. Morgen K, Kadom N, Sawaki L, Tessitore A, Ohayon J, McFarland H, et al. Training-dependent plasticity in patients with multiple sclerosis. *Brain*. (2004) 127:2506–17. doi: 10.1093/brain/awh266
63. Deluca J, Johnson SK. Cognitive impairments in multiple sclerosis. implications for rehabilitation. *NeuroRehabilitation*. (1993) 3:9–16. doi: 10.3233/NRE-1993-3404
64. Bensa C, Bodiguel E, Brassat D, Laplaud D, Magy L, Ouallet JC, et al. Recommendations for the detection and therapeutic management of cognitive impairment in multiple sclerosis. *Rev Neurol*. (2012) 168:785–94. doi: 10.1016/j.neurol.2012.02.009
65. Rosti-Otajärvi EM, Hämäläinen PI. Neuropsychological rehabilitation for multiple sclerosis. *Cochrane Database Syst Rev*. (2014) 1:CD009131. doi: 10.1002/14651858.CD009131.pub3
66. Dardiotis E, Nousia A, Siokas V, Tsouris Z, Andravizou A, Mentis AA, et al. Efficacy of computer-based cognitive training in neuropsychological performance of patients with multiple sclerosis: a systematic review and meta-analysis. *Mult Scler Relat Disord*. (2018) 20:58–66. doi: 10.1016/j.msard.2017.12.017
67. Amatya B, Khan F, Galea M. Rehabilitation for people with multiple sclerosis: an overview of Cochrane Reviews. *Cochrane Database Syst Rev*. (2019) 1:CD012732. doi: 10.1002/14651858.CD012732.pub2
68. Goverover Y, Chiaravalloti ND, O'Brien AR, DeLuca J. Evidenced-based cognitive rehabilitation for persons with multiple sclerosis: an updated review of the literature from 2007 to 2016. *Arch. Phys. Med. Rehabil*. (2018) 99:390–407. doi: 10.1016/j.apmr.2017.07.021
69. Sandroff BM, DeLuca J. Will behavioral treatments for cognitive impairment in multiple sclerosis become standards-of-care? *Int J Psychophysiol*. (2019). doi: 10.1016/j.ijpsycho.2019.02.010. [Epub ahead of print].
70. Castrén E, Heinonen T, Mäkinen K, Hämäläinen P, Kuusisto H. The rate of neuropsychological assessments in multiple sclerosis has increased-A retrospective study in a Finnish Central Hospital. *Acta Neurol Scand*. (2019) 141:156–61. doi: 10.1111/ane.13175
71. Amato MP, Langdon D, Montalban X, Benedict RH, DeLuca J, Krupp LB, et al. Treatment of cognitive impairment in multiple sclerosis: position paper. *J Neurol*. (2013) 260:1452–68. doi: 10.1007/s00415-012-6678-0
72. Prosperini L, Piattella MC, Giannì C, Pantano P. Functional and structural brain plasticity enhanced by motor and cognitive rehabilitation in multiple sclerosis. *Neural Plast*. (2015) 2015:481574. doi: 10.1155/2015/481574
73. Prosperini L, Di Filippo M. Beyond clinical changes: rehabilitation-induced neuroplasticity in MS. *Mult Scler*. (2019) 25:1348–62. doi: 10.1177/1352458519846096
74. Hallett M. Transcranial magnetic stimulation and the human brain. *Nature*. (2000) 406:147–50. doi: 10.1038/35018000
75. Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: state of the art 2008. *Brain Stimul*. (2008) 1:206–23. doi: 10.1016/j.brs.2008.06.004
76. Ayache SS, Chalah MA. The place of transcranial direct current stimulation in the management of multiple sclerosis-related symptoms. *Neurodegener Dis Manag*. (2018) 8:411–22. doi: 10.2217/nmt-2018-0028
77. Charvet L, Shaw M, Dobbs B, Frontario A, Sherman K, Bikson M, et al. Remotely supervised transcranial direct current stimulation increases the benefit of at-home cognitive training in multiple sclerosis. *Neuromodulation*. (2018) 21:383–9. doi: 10.1111/ner.12583
78. Leocani L, Chieffo R, Gentile A, Centonze D. Beyond rehabilitation in MS: insights from non-invasive brain stimulation. *Mult Scler*. (2019) 25:1363–71. doi: 10.1177/1352458519865734
79. Mattioli F, Bellomi F, Stampatori C, Capra R, Miniussi C. Neuroenhancement through cognitive training and anodal tDCS in multiple sclerosis. *Mult Scler*. (2016) 22:222–30. doi: 10.1177/1352458515587597
80. Iodice R, Manganelli F, Dubbioso R. The therapeutic use of non-invasive brain stimulation in multiple sclerosis—a review. *Restor Neurol Neurosci*. (2017) 35:497–509. doi: 10.3233/RNN-170735
81. Harel Y, Kalron A, Menascu S, Magalashvili D, Dolev M, Doniger G, et al. Cognitive function in multiple sclerosis: a long-term look on the bright side. *PLoS ONE*. (2019) 14:e0221784. doi: 10.1371/journal.pone.0221784
82. Luczynski P, Laule C, Hsiung GR, Moore GRW, Tremlett H. Coexistence of multiple sclerosis and Alzheimer's disease: a review. *Mult Scler Relat Disord*. (2019) 27:232–8. doi: 10.1016/j.msard.2018.10.109
83. Mandolesi G, Grasselli G, Musumeci G, Centonze D. Cognitive deficits in experimental autoimmune encephalomyelitis: neuroinflammation and synaptic degeneration. *Neurol Sci*. (2010) 31(Suppl.2):S255–9. doi: 10.1007/s10072-010-0369-3
84. Azevedo CJ, Overton E, Khadka S, Buckley J, Liu S, Sampat M, et al. Early CNS neurodegeneration in radiologically isolated syndrome. *Neurol Neuroimmunol Neuroinflamm*. (2015) 9:2:e102. doi: 10.1212/NXI.0000000000000102
85. Moccia M, Lanzillo R, Palladino R, Chang KC, Costabile T, Russo C, et al. Cognitive impairment at diagnosis predicts 10-year multiple sclerosis progression. *Mult Scler*. (2016) 22:659–67. doi: 10.1177/1352458515599075
86. Carotenuto A, Moccia M, Costabile T, Signoriello E, Paolicelli D, Simone M, et al. Associations between cognitive impairment at onset and disability accrual in young people with multiple sclerosis. *Sci Rep*. (2019) 9:18074. doi: 10.1038/s41598-019-54153-7

87. McKay KA, Manouchehrinia A, Berrigan L, Fisk JD, Olsson T, Hillert J. Long-term cognitive outcomes in patients with pediatric-onset vs adult-onset multiple sclerosis. *JAMA Neurol*. (2019). 76:1028–34. doi: 10.1001/jamaneurol.2019.1546
88. Johnen A, Bürkner PC, Landmeyer NC, Ambrosius B, Calabrese P, Motte J, et al. Can we predict cognitive decline after initial diagnosis of multiple sclerosis? Results from the German National early MS cohort (KKNMS). *J Neurol*. (2019) 266:386–97. doi: 10.1007/s00415-018-9142-y
89. Van Schependom J, Guldolf K, Béatrice D'hooghe M, Nagels G, D'haeseleer M. Detecting neurodegenerative pathology in multiple sclerosis before irreversible brain tissue loss sets in. *Transl Neurodegener*. (2019) 8:37. doi: 10.1186/s40035-019-0178-4
90. Fleischer V, Gröger A, Koirala N, Droby A, Muthuraman M, Kolber P, et al. Increased structural white and grey matter network connectivity compensates for functional decline in early multiple sclerosis. *Mult Scler*. (2017) 23:432–41. doi: 10.1177/1352458516651503
91. Wattjes MP, Rovira À, Miller D, Yousry TA, Sormani MP, de Stefano MP, et al. Evidence-based guidelines: MAGNIMS consensus guidelines on the use of MRI in multiple sclerosis–establishing disease prognosis and monitoring patients. *Nat Rev Neurol*. (2015) 11:597–606. doi: 10.1038/nrneurol.2015.157
92. Filippi M, Preziosa P, Rocca MA. Brain mapping in multiple sclerosis: lessons learned about the human brain. *Neuroimage*. (2019) 190:32–45. doi: 10.1016/j.neuroimage.2017.09.021
93. Brandstadter R, Katz Sand I, Sumowski JF. Beyond rehabilitation: a prevention model of reserve and brain maintenance in multiple sclerosis. *Mult Scler*. (2019) 25:1372–8. doi: 10.1177/1352458519856847
94. Messinis L, Kosmidis MH, Nasios G, Konitsiotis S, Ntoskou A, Bakirtzis C, et al. Do secondary progressive multiple sclerosis patients benefit from computer- based cognitive neurorehabilitation? *A randomized sham controlled trial. Mult Scler Relat Disord*. (2020) 39:101932. doi: 10.1016/j.msard.2020.101932

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

frontiers in Neurology

REVIEW
published: 22 April 2020
doi: 10.3389/fneur.2020.00251

The Potential Role of Neurophysiology in the Management of Multiple Sclerosis-Related Fatigue

Fioravante Capone[1,2]*, *Francesco Motolese*[1,2], *Emma Falato*[1,2], *Mariagrazia Rossi*[1,2] and *Vincenzo Di Lazzaro*[1]

[1] Unit of Neurology, Neurophysiology, Department of Medicine, Università Campus Bio-Medico di Roma, Rome, Italy, [2] NeXT: Neurophysiology and Neuroengineering of Human-Technology Interaction Research Unit, Campus Bio-Medico University, Rome, Italy

Fatigue is a very common symptom among people with multiple sclerosis (MS), but its management in clinical practice is limited by the lack of clear evidence about the pathogenic mechanisms, objective tools for diagnosis, and effective pharmacological treatments. In this scenario, neurophysiology could play a decisive role, thanks to its ability to provide objective measures and to explore the peripheral and the central structures of the nervous system. We hereby review and discuss current evidence about the potential role of neurophysiology in the management of MS-related fatigue. In the first part, we describe the use of neurophysiological techniques for exploring the pathogenic mechanisms of fatigue. In the second part, we review the potential application of neurophysiology for monitoring the response to pharmacological therapies. Finally, we show data about the therapeutic implications of neurophysiological techniques based on non-invasive brain stimulation.

Keywords: multiple sclerosis, fatigue, neurophysiology, non-invasive brain stimulation, TMS, tDCS

OPEN ACCESS

Edited by:
Moussa Antoine Chalah, Hôpitaux Universitaires Henri Mondor, France

Reviewed by:
Massimiliano Valeriani, Bambino Gesù Children Hospital (IRCCS), Italy
Mario Habek, University of Zagreb, Croatia

***Correspondence:**
Fioravante Capone
f.capone@unicampus.it

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *31 January 2020*
Accepted: *17 March 2020*
Published: *22 April 2020*

Citation:
Capone F, Motolese F, Falato E, Rossi M and Di Lazzaro V (2020) The Potential Role of Neurophysiology in the Management of Multiple Sclerosis-Related Fatigue. Front. Neurol. 11:251. doi: 10.3389/fneur.2020.00251

INTRODUCTION

Fatigue is a very common symptom in multiple sclerosis (MS) and produces significant detrimental effects on the quality of life (1). Despite its prevalence and impact, the management of fatigue in clinical practice is often challenging since the underlying pathophysiological mechanisms have not been well-elucidated (2), pharmacological treatments have limited efficacy (3), and fatigue assessment is commonly based exclusively on self-report questionnaires (4).

Although the advent of magnetic resonance imaging (MRI) significantly changed the overall management of MS, the role of neurophysiology remains of great importance in the functional evaluation of specific pathways such as visual, somatosensory, auditory, and motor systems and in the study of the central and the peripheral mechanisms of sensorimotor integration. Fatigue is a complex symptom including motor, cognitive, and psychological aspects, but through neurophysiological techniques, it is possible to evaluate mainly motor fatigue, from both research and clinical perspectives. Motor fatigue can be classified as central or peripheral. By definition, peripheral fatigue is the inability to generate force at the muscle level, while central fatigue refers to changes arising from the neural networks in the brain and the spinal cord, causing a lack of drive to the muscles.

The alterations occurring at the neuromuscular level cannot fully explain the phenomenon of fatigue (5), and in the last few years, different studies have speculated over the meaning and

magnitude of the contribution of the central nervous system (CNS). In particular, in MS, fatigue seems to arise from the disruption of a complex neural network involving the cerebral cortex, the thalamus, and the basal ganglia (6–8). Similarly also in other neurological conditions such as Parkinson's disease and stroke, different supraspinal structures are considered to be key players in fatigue generation (9).

In this scenario, neurophysiological techniques can play a decisive role in the assessment of the pathophysiology of MS-related fatigue, thanks to their ability to provide objective measures and to explore the peripheral and the central structures of the nervous system, with excellent time resolution. Besides that, various studies have also demonstrated good correlations between neurophysiological parameters and disability measures (10), highlighting the usefulness of neurophysiology in monitoring disease evolution and response to therapy.

Finally, several studies have evaluated the therapeutic implications of neurophysiological techniques based on non-invasive brain stimulation (NIBS) in different neuropsychiatric diseases such as stroke, depression, dementia, and movement disorders (11–13). In particular, in MS, promising results have been obtained in the treatment of disabling symptoms such as spasticity (14) and fatigue (15).

In this review, we will provide an outline of the current evidence about the potential role of neurophysiology in the management of MS-related fatigue. In the first part, we will describe the potential application of neurophysiological techniques for exploring the pathogenic mechanisms of fatigue. Then, we will report on the potential use of neurophysiology for measuring fatigue and monitoring the response to symptomatic therapies. In the third part, we will review the potential application of neuromodulation as an innovative treatment for fatigue. Eventually, we will discuss the limitations and the shortcomings of available data, highlighting the key challenges in the field and suggesting some directions for future research.

NEUROPHYSIOLOGY AS INVESTIGATING TOOL FOR THE PATHOGENIC MECHANISMS OF FATIGUE

During a physical effort, there is a progressive decline of firing rate of spinal motoneurons (16), but the significance of such phenomenon is not clear as it can be interpreted as exhaustion or as fatigue adaptation.

Most studies reported that MS patients present lower strength values of maximal voluntary contraction (MVC) in comparison to healthy subjects (17–20), and the decrease of these values is positively correlated with fatigue perception (21). The fall of muscle force (and MVC as well) could be related to a submaximal voluntary drive, which is known as central activation failure (CAF) (9). CAF can be evaluated by the twitch-interpolated technique, in which the subjects are asked to perform a MVC in a given muscle and an electrical stimulus is subsequently applied to the motor nerve supplying the tested muscle. If there is a further increase of muscle force after electrical stimulation, then the muscle's voluntary central drive was not at its maximum, thus demonstrating CAF. Using this technique, Steens et al. (22) showed a decrease of voluntary activation during fatiguing exercise in people with MS (PwMS) in comparison to healthy subjects, probably due to insufficient CNS compensatory mechanisms. The reduction of voluntary activation seems to be particularly important in the pathogenesis of fatigue in patients with secondary-progressive MS as compared to relapsing–remitting MS (23).

Electromyography (EMG) allows quantifying the reduction of amplitude or frequency of muscle action potentials (MAP) during a fatiguing task. Surface EMG (sEMG) is a non-invasive technique in which electrodes placed on the skin record electrical muscle activity (24, 25). In particular, the amplitude of the sEMG signal is considered as a measure of voluntary drive to peripheral structures (9). Muscle contraction is characterized by the progressive recruitment of different motor units, depending on their size, biochemical features, and fatigability (26, 27). The development of muscular fatigue produces specific changes in EMG signal, consisting in an initial increase and then in the decrease of MAP amplitude (28, 29), a reduction of median frequency of discharge, and a reduction of motor conduction velocity along fatigued muscle fibers (28, 30, 31).

These phenomena, also present in healthy subjects, are more evident in PwMS. For instance, Eken et al. found that prolonged walking produces a significant decrease of EMG median frequency with a corresponding increase of the root mean square of the EMG signal of the soleus muscle (32). Similar changes of EMG parameters have also been found in the upper limb by Severijns et al. (33) in a cohort of PwMS after a protocol of repetitive shoulder anteflexion movements. Interestingly, these changes in EMG parameters are present even without a clear performance decline and are not directly correlated with the level of perceived fatigue. These findings suggest that peripheral mechanisms cannot fully explain the development of fatigue and that central mechanisms could also be involved. In this regard, different neurophysiological methods can be used to study the contribution of CNS.

Electroencephalography (EEG) allows evaluating the role of cortico-cortical connections. Using this technique, Leocani et al. (34) investigated the correlation between fatigue severity [measured through the Fatigue Severity Scale (FSS) questionnaire] and EEG parameters consisting of event-related desynchronization (ERD) and event-related synchronization (ERS). They found that, in PwMS compared to healthy controls, FSS correlated positively with ERD over midline frontal structures during movement and inversely with contralateral sensorimotor ERS after movement. These findings suggest an overactivation of the frontal regions in fatigued patients, a possible expression of a compensatory mechanism for the subcortical dysfunction causing fatigue.

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique that can be used to explore the contribution of the different structures of the CNS to fatigue generation. Indeed single-pulse TMS allows evaluating the functionality of the corticospinal tract by recording the amplitude and the latency of motor-evoked potentials (MEP),

while paired-pulse TMS provides insight into the cortico-cortical connections. Moreover, repetitive TMS (rTMS) protocols are known to induce short- and long-term modifications of cortical excitability, thus reflecting plasticity changes at the cortical level.

In healthy subjects, MEP amplitude increases during a fatiguing exercise and reduces after its end (35). In MS patients, results are more variable because some studies reported a decrease of MEP amplitude similar to healthy subjects (36, 37), while others reported an increase (19, 38) or no changes (39). Also, in the premovement phase, a significant lack of MEP facilitation after a sustained motor task was shown in fatigued PwMS compared to controls and not-fatigued patients (40, 41), suggesting a disruption of the brain networks involved in motor preparation which has been correlated to structural and functional changes in frontal-thalamic pathways (41).

Different paired-pulse TMS studies have demonstrated, in healthy subjects, physiological modifications of cortical excitability as a result of fatigue development. Paired-pulse TMS protocols are used to test different cortical circuits (42) and include short-interval cortical inhibition (SICI) (43), a protocol related to inhibitory gamma-aminobutyric acid (GABA)-A interneurons, in which a subthreshold conditioning first pulse inhibits the response to a suprathreshold second pulse delivered 1–5 ms later (44); intracortical facilitation (ICF) (45), linked to glutamatergic intracortical circuits in which a subthreshold conditioning first pulse enhances the response to a suprathreshold second pulse delivered 7–20 ms later (46); and late intracortical inhibition (LICI) (47), mediated by GABA-B receptors in which two suprathreshold pulses at long-interstimulus intervals of 50–200 ms are delivered (48). Benwell et al. (49) showed that SICI initially increases and then decreases as force declines during a fatiguing exercise involving the first dorsal interosseous (FDI) muscle. Similarly, Maruyama et al. (50) found a transient reduction of SICI in FDI muscle after isometric contractions, while there was no change in ICF. By contrast, Hunter et al. (51) likewise found a reduction of SICI, while ICF decreased during a sustained submaximal voluntary muscle contraction. Besides that, changes of ICF or SICI seem to depend also on the type of fatiguing motor task used in the experimental protocol—for instance, being different during handwriting compared to isometric finger abduction (52).

In PwMS, different alterations in cortical excitability parameters have been described. Liepert et al. (37) found that, compared to healthy controls and to PwMS without fatigue, SICI was reduced in PwMS with fatigue, already at baseline, before the fatiguing exercise. In contrast, Morgante et al. (40) found similar values of SICI and ICF in PwMS with and without fatigue and in healthy controls, while Chalah et al. found a significant reduction of SICI in non-fatigued compared to fatigued PwMS and no significant difference in ICF and other TMS measures (53).

Another neurophysiological measure which can be assessed through TMS is the cortical silent period (CSP) that is an interruption of the voluntary muscle contraction after a TMS pulse over the contralateral motor cortex and is thought to be mediated by GABA-B inhibitory neurotransmission, (54). CSP duration in PwMS predicted fatigue and was associated with poor cardiovascular fitness (55).

Several studies have investigated the changes of cortical plasticity of PwMS through rTMS protocols (56, 57), but only a few of them have explored their role in fatigue pathogenesis.

Morgante et al. (40) found that PwMS have reduced plasticity demonstrated by the lack of MEP increase after the 5-Hz rTMS protocol, without any difference between fatigued and not-fatigued patients. Conte et al. (58) found instead that, during an attention-demanding task, the response to 5-Hz rTMS and paired associative stimulation (PAS)—a neuromodulatory protocol consisting of repetitive peripheral nerve stimulation combined with TMS over the contralateral motor cortex (59)—significantly differs between PwMS with or without fatigue. Indeed in fatigued patients both PAS and 5-Hz stimulation did not produce the expected changes in cortical excitability, while in not-fatigued patients they both increased the MEP response, although less efficiently than in healthy subjects.

TMS techniques do not allow a complete evaluation of brain subcortical structures, the role of which seems to be crucial in fatigue generation. In a recent study, Capone et al. (60) evaluated how high-frequency oscillations (HFOs)—a burst of fast oscillations that overlies the cortical response of median nerve somatosensory-evoked potentials—are influenced by a fatiguing exercise in a cohort of 15 PwMS and 15 healthy controls. They showed a significant change of the early component of HFOs, reflecting the possible primary role played by the thalamus in the pathogenesis of MS-related fatigue, while the latter component reflects that the cortico-cortical network activity in the somatosensory cortex was not modified significantly. Furthermore, increasing evidence from neuroimaging studies is supporting the hypothesis that the thalamus is a key player in fatigue generation (6).

Fatigue is a complex symptom involving both cognitive and motor domains and multiple factors, in addition to sensorimotor dysfunction as assessed by EEG and EPs, which can contribute to its pathogenesis and/or exacerbate its manifestations (demographics, comorbidity, genetics, diet, exercise, depression, cognitive impairment, pain, and sleep disorders) (6). Neurophysiology can also play an important role in defining and quantifying some of these factors. For instance, event-related potentials (ERP) could be a useful tool to investigate the mechanisms involved in the pathogenesis of cognitive fatigue.

Pokryszko-Dragan et al. found that fatigued PwMS have worse cognitive performances and delayed latency in the P300 component of the auditory ERP and also in the early stage of the disease. These results were confirmed by Chinnadurai et al. (61) in a sample of 50 PwMS using a modified version of auditory ERP. However, a recent study by Lazarevic et al. (62) did not find any effect of depression and fatigue on the ERP parameters. Thus, further research is needed to clarify the role of ERP in the assessment of cognitive impairment in PwMS.

It has been demonstrated that sleep disorders such as obstructive sleep apnea (63), restless leg syndrome (64, 65), periodic limb movements (66), and rapid eye movement behavior disorders (67) are more frequent in PwMS than in the general population and can contribute to the development of motor (2) and cognitive fatigue (68). In all these disorders, overnight

polysomnography is essential to make a diagnosis and to quantify the consequent reduction of sleep efficiency (69).

Moreover, the disease itself can produce pathological and functional modifications in the CNS that alter the restorative sleep capacity and thus exacerbate fatigue perception. This phenomenon was investigated by Bridoux et al. using TMS for assessing the reduction of MEP amplitude induced by an exercise (post-exercise corticomotor depression or PECD). They demonstrated that, in healthy subjects, sleep enhances recovery from PECD, while in PwMS, the restorative effect of sleep is reduced or lost (70).

Autonomic dysfunction is very common among PwMS and can occur since the earliest stages of the disease. It is mainly caused by demyelinating lesions located in the periventricular region of the fourth ventricle, in the brainstem, and in the spinal cord (69). Autonomic dysfunction can produce different symptoms affecting the bowel, the bladder, the heart, and the blood vessels.

The functionality of the autonomic nervous system can be tested by the Quantitative Sudomotor Axon Reflex testing (71) and the study of cardiovascular parameters such as blood pressure and heart rate response to Valsalva maneuver, heart rate variability during deep breathing, and blood pressure and heart rate changes during tilt test (72).

In particular, cardiac autonomic dysfunction has been associated to fatigue in PwMS (73), but the mechanisms and significance of this association remain unclear.

Some authors have hypothesized that MS-related fatigue is caused by a sympathetic vasomotor dysfunction with a normal parasympathetic activity (74–76).

On the contrary, other studies found that fatigued PwMS have a reduction in vagal activity compared to controls (77–79).

Recent evidence suggests that pupillometry could be an alternative method to evaluate the involvement of the autonomic nervous system in PwMS. Indeed the pupil size depends on the balance between the sympathetic and parasympathetic components of the autonomic nervous system. For instance, de Rodez Benavent et al. (80) investigated the changes in pupil size during problem-solving in MS patients (with and without fatigue) vs. controls. They found that MS-related changes in cognition and fatigue could be associated with changes in the autonomic regulation of task-related pupillary responses.

Taken together, the neurophysiologic data demonstrated that MS-related fatigue seems to have a central origin. The changes in EMG parameters, described in MS patients (32, 33), are thought to be more a consequence of alterations in CNS structures rather than a primary determinant of fatigue. However, it cannot completely be ruled out that such changes could be the epiphenomenon of peripheral alterations occurring at the neuromuscular level.

Neuroimaging studies (60, 81, 82) demonstrated that the main pathogenic substrate of MS-related fatigue could be a dysfunction of the circuits between the thalamus, the basal ganglia, and the cortex, and neurophysiological findings support this hypothesis. Indeed single-pulse TMS studies demonstrated that in MS patients the pathogenesis of fatigue is not driven by mechanisms directly related to corticospinal functioning but is due to alterations in structures located upstream to the primary motor cortex (39). In particular, both EEG (34) and TMS studies (37, 40, 58) pointed out the role of cortical areas involved in movement preparation and attention. For instance, Sandroni et al. (83) found that, in PwMS, fatiguing tasks are associated with a change in ERP without significant modifications in MEP parameters, thus suggesting that fatigue affects neural processes acting after stimulus evaluation and before the activation of the primary motor cortex.

More recently, Capone et al. (60) explored the contribution of the thalamus by means of HFOs obtained from the median nerve SEP, demonstrating that a dysfunction of the thalamo-cortical axons contributes to fatigability in MS patients.

Although CNS functional alterations are consistently reported by neurophysiological studies, their significance remains largely unknown because they were considered by some authors as pathogenic factors (40) and by others as the epiphenomena of adaptive processes (60). According to the first hypothesis, neurophysiologic techniques measure the change in the activity of CNS networks caused by the MS-related damage of gray and white matter. On the other side, according to the alternative hypothesis, this damage produces compensatory/adaptive mechanisms that can be recorded by means of neurophysiological techniques.

More broadly, several structural and functional abnormalities in various cortico-subcortical neural networks (e.g., fronto-striatal network, cortico-striato-thalamo-cortical loop) occur during MS as a result of inflammation, neurodegeneration, and compensatory neuroplasticity processes. From this perspective, the development of fatigue could depend on the dynamic balance between damage and restorative processes during the disease's course (8). Indeed the latter can be predominant in the initial phase of the disease, thus masking the clinical occurrence of fatigue, while, later on, the damage could prevail so that patients experience clinically relevant fatigue. Accordingly, the heterogeneity in the results of neurophysiological studies can depend on the stage of the disease in which the recording has been done.

Interestingly, the neurophysiological markers of fatigue at different levels, such as changes in EMG parameters (33), in HFO features (60), or in cortical plasticity (40), can also be observed in MS patients without fatigue. This finding could suggest that an impairment in fatigability mechanisms (expressed by neurophysiological alterations) does exist in MS since the earliest phases of the disease, independently from the level of fatigue in everyday life measured through questionnaires. This is not surprising because fatigue is a multifactorial and complex symptom, and different factors, in addition to thalamo-cortical dysfunction, could be necessary to make it clinically relevant.

MS can cause extensive damage of the CNS, so it is not surprising that autonomic nervous system involvement or subtle alterations of cognitive functioning may occur at any stage of the disease. Thus, these are other factors that need to be considered as potential players in fatigue generation, but evidences are not unambiguous. Sleep disorders should also be taken into account since the impairment of a restorative process can exacerbate—or even be one of the main generators— fatigue (2, 68, 70).

Longitudinal studies involving patients at different stages of the disease (from clinically isolated syndrome to advanced progressive MS) and investigating possible factors involved in fatigue perception (such as genetics, comorbidity, cognitive impairment, depression, and sleep disorders) could contribute to corroborate such hypothesis. In **Table 1**, we have summarized the studies that have used neurophysiological techniques for investigating fatigue pathogenesis.

NEUROPHYSIOLOGY FOR MONITORING THE RESPONSE TO THERAPIES FOR FATIGUE

The most frequently used pharmacological treatments for fatigue are amantadine, 4-aminopyridine, and modafinil. The non-pharmacological interventions include physical (e.g., aerobic exercises, resistance training, yoga, and tai-chi) and psychological/cognitive approaches (e.g., cognitive behavioral therapy, education programs, and mindfulness interventions). However, evidence supporting the efficacy of these interventions is still preliminary and, sometimes, conflicting (87).

Amantadine is an antiviral agent firstly introduced to prevent and treat flu viruses. Animal models have shown that amantadine induces the release of dopamine from nerve endings (88). Moreover, one clinical trial has shown an increased level of beta-endorphin and beta-lipoprotein after amantadine assumption, with clear clinical improvement (89). The real mechanism of action of amantadine as fatigue therapy is not yet clear, but the fact that amantadine acts as a dopaminergic factor supports the dopamine imbalance theory for fatigue generation (90). One relevant study, addressing the neurophysiological effects of amantadine in MS-related fatigue, was conducted by Santarnecchi et al. (91). They found that chronic treatment with this drug improves clinical fatigue (assessed through questionnaires) and restores GABAergic inhibitory mechanisms in the motor cortex of PwMS, as indicated by the normalization of CSP in basal condition and by the reduction of CSP duration after a fatiguing task. Reis et al. (92) evaluated the effect of a single dose of amantadine on human motor cortex excitability in healthy subjects. They showed that a single dose of amantadine significantly decreases ICF and increases LICI in the motor cortex. MEP recruitment curves, motor thresholds, and duration of CSP remained unchanged after treatment. These data suggested that a single dose of amantadine is able to modulate motor cortex excitability, possibly involving GABAergic and glutamatergic neurotransmission.

Another drug, tested for MS-related fatigue, was modafinil, a central alpha-adrenergic agonist approved for the treatment of attention-deficit hyperactivity disorder and narcolepsy. Lange et al. (93) reported a significant improvement of fatigue questionnaire scores and in the nine-hole peg test, after modafinil administration, in a group of 21 PwMS. Furthermore, they tested different TMS protocols before and after 8 weeks of treatment, showing an increase of MEP size by paired pulse TMS, in the modafinil group.

Nagels et al. (94) evaluated visual- and auditory-evoked potentials (EP) for predicting the response to modafinil treatment (100 mg, once daily, for 4 weeks), in 33 PwMS with fatigue. They found that the latency of auditory P300 predicted the treatment response with a good specificity and sensitivity. In particular, a shorter latency at baseline was associated with a better response to modafinil treatment.

In order to better clarify the mechanisms of action of modafinil in fatigue relief, Niepel et al. (76) investigated the effect of a single dose (200 mg) of modafinil on measures of alertness and autonomic function in fatigued PwMS compared to not-fatigued PwMS and healthy controls.

They found that fatigued patients had a reduced level of alertness and cardiovascular sympathetic activation compared to the other two groups, and modafinil was able to reverse these deficiencies. On the basis of these findings, they hypothesized that the anti-fatigue effect of modafinil was related to the activation of the noradrenergic locus coeruleus (76).

Despite these interesting data, at present, there is no indication, in clinical practice, for the use of modafinil for fatigue relief.

Potassium channel blockers—e.g., 4-aminopyridine (4-AP)—belong to a group of drugs able to restore conduction propriety in demyelinating axons as shown in animal models (95). Different trials have also explored the central effect of 4-AP, speculating on a potential role in optimizing neurotransmitter release at the synaptic level (dopamine, acetylcholine, noradrenaline, and serotonin). This latter hypothesis is supported by the observation of an increase BOLD signal during a motor task following a 3,4-diaminopyridine administration compared with a placebo dose assumption (96).

Sheean et al. (97) evaluated changes in TMS-evoked corticospinal excitability parameters in eight PwMS with fatigue before and after treatment with 3,4-diaminopyridine. The motor performance of adductor pollicis muscle was evaluated by TMS, rapid voluntary movements, and a fatiguing exercise test consisting of a sustained isometric contraction. After 3 weeks, fatigue was significantly reduced but neurophysiological parameters (central motor conduction time and MEP amplitude) did not change in the treated patients compared to the untreated ones. These findings suggest that the effect of 3,4-diaminopyridine on fatigue could be linked with mechanisms and structures other than corticospinal tract functionality. Moreover, methodological factors should be considered in the interpretation of these results. Indeed only upper limbs spared from the disease were evaluated, thus representing a major limitation of the study.

More recently, Marion et al. designed a randomized double-blind placebo-controlled trial to investigate the effect of modified-release 4-aminopyridine (fampridine) on upper limb function, fatigue, and several neurophysiological parameters such as visual-evoked potentials (latency and amplitude), somatosensory-evoked potentials (latency and amplitude), motor-evoked potentials (latency), central motor conduction time, resting motor threshold, MEP recruitment curves, and

TABLE 1 | Neurophysiological studies exploring the pathogenic mechanisms of fatigue in PwMS.

References	Neurophysiologic technique	Sample size	Sample composition	Main findings
Steens et al. (22)	EMG	20 PwMS + 20 HCs	20 patients (RR); age range: 20–58 years; EDSS <5.5	Positive correlation between fatigue perception and the decline of MCV during a sustained contraction
Rice et al. (17)	EMG	4 PwMS + 16 HCs	4 patients (SP, RR); age range: 28–53 years; mean EDSS 4.6	PwMS present lower values of MVC
Sheean et al. (18)	EMG	21 PwMS + 19 HCs	21 patients (RR; SP, PP); age range: 26–55 years; mean EDSS: 2–8	PwMS present lower values of MVC
Perretti et al. (19)	MEP	41 PwMS	41 patients (RR), on IFN b1a treatment; age range: 30.7 ± 8.8; EDSS: 3.2 ± 0.5; divided into fatigued and not-fatigued	MS patients do not have TMS MEP depression following fatiguing exercise, while post-exercise MEP facilitation was similar to that seen in normal subjects
Steens et al. (22)	EMG	20 PwMS + 20 HCs	20 patients; age range: 21–58 years; EDSS ≤ 5	Decrease of voluntary activation during fatiguing exercise in PwMS in comparison to HC
Wolkorte et al. (23)	EMG	45 PwMS + 25 HCs	45 patients (RR, SP); age range: 20–65 years; EDSS: 0–7	Compared to controls, the SPMS patients had reduced voluntary activation during brief and sustained contractions.
Eken et al. (32)	EMG	8 PwMS + 10 HCs	8 patients (RR, SP, PP); age range: 49±9 years; EDSS: 1–6	Prolonged walking produces a significant decrease of EMG median frequency and an increase of root mean square EMG signal of the soleus muscle
Severijns et al. (33)	EMG	16 PwMS + 16 HCs	16 patients (RR, SP, PP); age range: 55 ± 8 years; mean EDSS: 6; divided into fatigued and not-fatigued	PwMS with hand grip weakness, experience a larger increase in fatigue compared to PwMS with normal hand grip strength
Leocani et al. (34)	EEG	33 PwMS + 14 HCs	33 patients; EDSS < 1.5; divided into fatigued (age: 33 ± 8 years) and not-fatigued (age: 32 ± 6 years)	In PwMS, FSS correlated positively with ERD over midline frontal structures during movement and inversely with contralateral sensorimotor ERD after movement
Petajan and White (36)	MEP	32 PwMS + 10 HCs	32 patients; divided into 2 subgroups: patients without weakness of upper limbs (age: 44 ± 10.3 years) and patients with weakness of upper limbs (age: 42.9 ± 9.9 years)	Decrease of MEP amplitude similar to HCs
Liepert et al. (37)	MEP	16 PwMS RR + 6 HCs	16 patients, divided in 2 subgroups: fatigued (FSS > 4, mean EDSS: 3.1); not-fatigued (FSS < 4, mean EDSS: 2.9)	Decrease of MEP amplitude similar to HC; in fatigued patients, SICI was reduced at baseline
Thickbroom et al. (38)	MEP	10 PwMS + 13 HCs	10 patients (RR); age range: 33–64 years; EDSS ≤ 4; MRC grade ≥ 4/5	Increase of MEP amplitude in PwMS compared to HC
Mordillo-Mateos et al. (39)	MEP	17 PwMS + 16 HCs	17 patients (RR; SP); mean age: 36.3 ± 9.5 years; mean EDSS: 5	No changes in MEP amplitude in the two groups
Morgante et al. (40)	MEP	33 PwMS	33 patients (RR), divided into 2 subgroups: fatigued (mean age 38 ± 9.4 years; mean EDSS 1.6 ± 0.6) and not-fatigued (mean age 41.1 ± 10.9 years; mean EDSS 1.8 ± 0.6)	PwMS with fatigue lacked pre-movement facilitation compared to PwMS without fatigue and HC
Conte et al. (58)	5Hz rTMS, PAS	25 PwMS + 18 HCs	25 patients (RR); EDSS < 3.5; divided into 2 subgroups, fatigued (mean age 41.3 ± 7.7 years; mean EDSS 1) and not-fatigued (mean age 38.3 ± 8.4 years; mean EDSS 1.1)	In non-fatigued patients, PAS and rTMS increased the MEP response; in fatigued patients, they did not produce changes in cortical excitability
Capone et al. (60)	SEP, HFO	15 PwMS + 15 HCs	15 patients (RR); mean age: 42.1 years; mean EDSS 1	Fatiguing task induces a change in the early component of HFOs in PwMS
Russo et al. (41)	MEP	24 PwMS + 10 HCs	24 patients (RR), age range: 18–65 years; EDSS ≤ 2.5	Premovement facilitation is reduced in fatigued PwMS
Russo et al. (84)	MEP	30 PwMS	30 patients (RR); mean age: 24–63 years; EDSS < 3.5; divided into 2 subgroups, fatigued and not-fatigued	Fatigue is associated with a disruption of brain networks involved in motor preparation processes, depending on frontal-thalamic pathways
Chalah et al. (53)	MEP	38 PwMS	38 patients (RR, PP, SP); age range: 34–67 years; EDSS: 3–6.5; divided into 2 subgroups, fatigued and not-fatigued	Fatigued patients had higher depression, anxiety, alexithymia scores, higher SICI, larger caudate nuclei, and smaller left parietal cortex.
Chaves et al. (55)	MEP	82 PwMS	92 patients (RR; PP, SP); mean age: 47.40 ± 10.2 years; EDSS 2.04 ± 1.	Longer CSP predicted worsened fatigue in PwMS
Pokryszko-Dragan et al. (85)	ERP	86 PwMS + 40 HCs	86 patients (CIS; RR; SP); age range: 19–60 years; EDSS: 1–6.5; divided into 3 groups: not fatigued, moderately fatigued, severely fatigued	Fatigued PwMS have worse cognitive performances and delayed latency in P300 component of auditory ERP

(Continued)

TABLE 1 | Continued

References	Neurophysiologic technique	Sample size	Sample composition	Main findings
Pokryszko-Dragan et al. (86)	ERP	44 CIS + 45 HCs	44 patients (CIS); age range: 21 – 48 years; EDSS: 1–2	N200 latency was correlated with fatigue.
Chinnadurai et al. (61)	ERP	50 PwMS + 50 HCs	50 patients (RR; PP; SP); age range: 13–66 years; EDSS: 1–9	Clinical measures of cognitive fatigue were correlated with the neurophysiological measures (ERP)
Lazarevic et al. (62)	ERP	81 PwMS + 32 HCs	81 patients (RR); age: 41.09 ± 8.72 years; EDSS: 0–7; divided in two groups: fatigued and not fatigued	Depression and fatigue have no effect on ERP amplitude and latency
Bridoux et al. (70)	MEP	30 PwMS + 15 HCs	12 fatigued patients (RR; SP); mean age: 44 ± 3 years; EDSS: 1–3.5	In PwMS, sleep does not enhance motor recovery from PECD following a fatiguing exercise
Lebre et al. (74)	ANS testing	50 PwMS	50 patients (RR); mean age 37 years; EDSS < 3.5; divided in two subgroups: fatigued and not-fatigued	Loss in the capacity to increase the blood pressure in patients with fatigue, suggesting a sympathetic dysfunction
Flachenecker et al. (75)	ANS testing	60 PwMS + 36 HCs	60 patients (RR); mean age 41.5 ± 9.9 years; mean EDSS 3.0; divided in two subgroups: fatigued and not-fatigued	The median HR response to standing (HR-Post30/15) was significantly reduced, and BP-Grip tended to be lower in pwMS compared to HCs.
Niepel et al. (76)	Sleep study	26 PwMS + 9 HCs	26 patients (RR; SP; PP); divided in 2 subgroups, fatigued (FSS > 5; age range 49.4 ± 9.2 years) and not-fatigued patients (FSS < 4.0; age range 41.8 ± 13.1 years)	Fatigue patients showed evidence of reduced level of alertness on a number of subjective and objective measures of alertness, in contrast to non-fatigued MS patients and HCs
Keselbrener et al. (77)	ANS testing	10 PwMS + 10 HCs	10 patients; age: 22–58 years; FSS > 3.5	Fatigued PwMS showed a reduction in vagal activity which was more marked than in the control subjects
Heesen et al. (78)	ANS testing	23 PwMS + 25 HCs	23 patients (RR; SP); mean age: 40.13 ± 2.23 years; mean EDSS 2.36 ± 0.36. 14 patients on DMD (8 interferon, 5 glatiramer acetate, 1 azathioprine)	Cognitive stress induces IFNγ production in HC but not in MS patients with fatigue. Reduced cardiac response might indicate an autonomic dysfunction in PwMS.
Sander et al. (79)	ANS testing	53 PwMS	53 patients (RR, SP, PP); mean age: 50.1 ± 8.7 years; mean EDSS 3.3 ± 1.7	Reduced responsiveness and high- and very-low-frequency components of HR variability, indicating an increased parasympathetic activity
de Rodez Benavent et al. (80)	ANS (pupillary response)	49 PwMS + 46 HCs	49 patients (RR); age range: 18–50 years; mean EDSS 1.9 ± 0.8	MS-related changes in cognition and fatigue could be associated with changes in the autonomic regulation of task-related pupillary responses

EMG, electromyography; PwMS, people with multiple sclerosis; HCs, healthy controls; RR, relapsing–remitting; PP, primary progressive; SP, secondary progressive; EDSS, expanded disability status scale; MVC, maximum voluntary contraction; MEP, motor-evoked potentials; IFN, interferon; TMS, transcranial magnetic stimulation; EEG, electroencephalography; ERD, event-related desynchronization; FSS, fatigue severity scale; SICI, short-interval intracortical inhibition; PAS, paired associative stimulation; rTMS, repetitive transcranial magnetic stimulation; SEP, somatosensory-evoked potentials; HFO, high-frequency oscillations; CSP, cortical silent period; CIS, clinically isolated syndrome; PECD, post-exercise cortical depression; ERP, event-related potentials; ANS, autonomic nervous system; HR, heart rate; BP, blood pressure; MS, multiple sclerosis.

paired-pulse TMS protocols. They found that fampridine (10 mg bd, for eight consecutive weeks) did not produce significant changes in upper limb function, fatigue, and neurophysiological parameters (98).

Over the last years, various studies have demonstrated that neurophysiology can be helpful in measuring and predicting response to treatment. However, the results are not definitive since data are scarce and sometimes not conclusive. Studies greatly differ from each other in variables such as outcome measures, treatment and follow-up duration, neurophysiological techniques, and clinical features of patients. Moreover, to the best of our knowledge, no study has evaluated, through neurophysiological tools, the effectiveness of non-pharmacological interventions such as physical, psychological, and cognitive approaches. Anyway, it still seems reasonable to assume that neurophysiology can have a role in monitoring the response to fatigue treatment, and more studies on the matter are warranted.

In **Table 2**, we have summarized the studies that have used neurophysiological techniques for monitoring the treatment for fatigue in PwMS.

NEUROPHYSIOLOGY AS INNOVATIVE TREATMENT FOR FATIGUE IN MS PATIENTS

Neurophysiological studies are being carried out not only to identify objective and measurable markers of fatigue, as previously illustrated, but also to find neuromodulation protocols able to reduce this disabling symptom.

NIBS approaches are playing a major role in this research setting, following a large neurophysiological evidence of central abnormalities in PwMS with fatigue (34, 39, 56, 99).

In **Table 1**, the results of a MEDLINE research on sham-controlled NIBS studies for the treatment of fatigue in PwMS

TABLE 2 | Neurophysiological studies for monitoring response to therapies for fatigue in PwMS.

References	Therapy	Neurophysiologic technique	Sample size	Sample composition	Main findings
Santarnecchi et al. (91)	Amantadine	MEP, EMG for CSP study	10 PwMS + 10 HCs	10 patients (RR; SP); age range: 24–44 years; mean EDSS: 2.1 ± 1.4	Normalization of CSP in basal condition and a reduction of CSP duration after the fatiguing task
Reis et al. (92)	Amantadine, single dose	MEP, EMG for CSP, SICI, LICI	14 HCs	14 healthy volunteers; mean age: 25 ± 2.8 years	A single dose of amantadine was able to modulate motor cortex excitability (decreases ICF and increases LICI in M1)
Lange et al. (93)	Modafinil, 100 mg/day for the first week and 200 mg/day for subsequent 7 weeks vs. placebo	MEP	21 PwMS	21 patients, FSS ≥ 36, EDSS < 7.0; divided into 2 subgroups: treated (mean age: 42.6 ± 9.7 years; mean EDSS; 3.1 ± 0.6) and placebo (mean age: 44.1 ± 12.1 years; mean EDSS: 3.2 ± 1.1)	Increase MEP size by paired pulse TMS in the modafinil group
Nagels et al. (94)	Modafinil, 100 mg, once daily, for 4 weeks	ERP	33 PwMS	33 fatigued patients (RR; SP; PP); mean age: 43 ± 2 years; mean EDSS: 5	A shorter P300 latency at baseline was associated with a better response to modafinil treatment
Sheean et al. (97)	3,4- diaminopyridine	MEP	8 PwMS	8 patients (RR; SP; PP); mean age: 39 years; mean EDSS: 6	After treatment, fatigue was significantly reduced but the neurophysiological parameters (central motor conduction tip and MEP amplitude) did not change
	4-AP vs. fluoxetine	SEP, MEP	60 PwMS	60 patients (RR); age range: 18–50 years; mean EDSS: 5.5; divided into 2 subgroups: fatigued (mean EDSS: 3.3 ± 2.5) and not-fatigued (mean EDSS: 3.1 ± 2.3)	Significant reduction of the fatigue questionnaire scores, with a greater reduction for the 4-AP subgroup
Marion et al. (98)	4-aminopyridine, 10 mg bd, for 8 consecutive weeks vs. placebo	VEP, SEP, MEP	40 PwMS	40 patients (RR; SP; PP); mean age: 52 years; mean EDSS: 6.0	Fampridine did not produce significant changes in upper limb function, fatigue, and neurophysiological parameters

MEP, motor evoked potentials; EMG, electromyography; CSP, cortical silent period; PwMS, people with multiple sclerosis; HCs, healthy controls; RR, relapsing–remitting; PP, primary progressive; SP, secondary progressive; EDSS, expanded disability status scale; SICI, short interval intracortical inhibition; LICI, long-interval intracortical inhibition; TMS, transcranial magnetic stimulation; ERP, event-related potentials; SEP, somatosensory-evoked potentials; VEP, visual-evoked potentials.

is presented, and the stimulation parameters are described for each study.

Transcranial direct current stimulation (tDCS) is the NIBS technique mostly used so far (cf. **Table 1**). It is classically assumed that tDCS can modulate human brain activity with effects that could outlast the period of stimulation by inducing a subthreshold shift of the resting membrane potential toward depolarization (anodal tDCS) or hyperpolarization (cathodal tDCS) (15). Beyond local effects, connectional (axonal) and non-neuronal effects have also been described (15). The tDCS mechanisms of action are still incompletely understood; an effect on calcium-dependent synaptic plasticity of glutamatergic neurons and a local reduction in GABA neurotransmission have been hypothesized (15).

Anodal tDCS applied to the motor cortical areas reduced motor fatigue in healthy subjects (100, 101). In patients with MS-related fatigue, anodal tDCS has been used with variable effects, depending on the parameters of stimulation and the clinical characteristics of the patients included in the studies.

As shown in **Table 1**, different targets have been stimulated by anodal tDCS. The evidence of functional alterations in the frontal areas in PwMS with fatigue (8) focused the attention of some researchers on the stimulation of the left dorsolateral prefrontal cortex (DLPFC).

Among these studies, negative results were reported by Saiote et al. (102) and Ayache et al. (103). Some methodological factors such as the wash-out duration and the stimulation intensity (102), the stimulation duration, and the heterogeneity of the population included (103) could have played a role in these results. Other three studies reported positive results on fatigue after anodal tDCS was applied over the left DLPFC (104–106). Among these, worthy of note are the use of a remotely supervised tDCS system in combination with a computer-based cognitive training (105) and the use of objective outcome measures, such as the P300 evoked potential and the reaction time (106). The application of anodal tDCS to the motor cortex bilaterally (107) and to the right parietal cortex (108) also gave a preliminary evidence of efficacy.

The group of Tecchio et al. focused on a personalized anodal tDCS approach targeting the whole-body primary somatosensory areas (S1) bilaterally, following the evidence of S1 reduced excitability and M1 hyperexcitability in PwMS with fatigue (109–112). They used a tailored procedure with personalized electrodes based on the patients' brain MRI located in place through an

TABLE 3 | Sham-controlled NIBS studies for the treatment of MS-related fatigue.

References	Anode: location dimensions	Cathode: location dimensions	Stim duration stim intensity	Efficacy	Fatigue evaluation methods	Study design	Sample size and MS subtype	Sample composition (values expressed as mean ± SD, when available)	Adverse effects
1) Anodal Tdcs									
Saiote et al. (102)	Left DLPFC 5 × 7 cm	Right forehead 6 × 15 cm	20 min/day, 5 days 1 mA	No	- FSS - MSFSS - MFIS	Crossover, sham-controlled (2-week wash-out)	13 RR	Clinically definite MS (121) Age: 46.9 ± 6.8 EDSS: 3.5 ± 4.0 FSS: 5.67 ± 2.47 MFIS: 47 ± 31	Tingling, light headache
Ayache et al. (103)	Left DLPFC 25 cm^2	Right supraorbital region 25 cm^2	20 min/day, 3 days 2 mA	No	MFIS (secondary outcome)	Crossover, sham-controlled (3-week wash-out)	16 (11RR, 4SP, 1PP)	Clinically definite MS (121) and history of neuropathic pain with VAS >40 Age: 48.9 ± 10 EDSS: 4.25 ± 1.4 MFIS: 52.6 ± 12.2	Insomnia, nausea, severe headache, phosphenes
Chalah et al. (104)	a) Left DLPFC 25 cm^2 b) Right PPC 25 cm^2 in different blocks	a) Right supraorbital region 25 cm^2 b) Cz (EEG 10-20 system) 25 cm^2	20 min/day, 5 days 2 mA	a) Yes (on FSS and on MFIS physical and psychosocial subscales) b) No	- FSS - MFIS - VAS	Crossover. sham-controlled (3-week wash-out)	10 (9 RR, 1 SP)	Clinically definite MS (121) Age: 40.50 ± 11.18 EDSS: 2.3 ± 2.5 FSS: 6.5 ± 3.8	a) None b) Insomnia, headache
Charvet et al. (105)	Left DLPFC 5 × 5 cm	Right DLPFC 5 × 5 cm	Remotely supervised tDCS combined with computer-based cognitive training 20 min/day, 20 days over 4 weeks From 1.5 to 2 mA	YES	- FSS - PROMIS-fatigue short form - VAS	Randomized, sham-controlled	27 (15 active of which 40% RR, 12 sham of which 58% RR)	Clinically definite MS Active group ($n = 15$): - age: 44.8 ± 16.2 - EDSS: 6.0 (range 0.0–7.0) - FSS (%clinical fatigue): 50 Sham group ($n = 12$): - age: 43.4 ± 16.2 - EDSS: 3.5 (range 0.0–8.5) - FSS (%clinical fatigue): 76	Tingling, itching, burning, head pain, difficulty concentrating
Fiene et al. (106)	Left DLPFC 5 × 5 cm	Right shoulder 5 × 7 cm	Single session of 27.29 ± 1.15 min (10 min tDCS only, 20 min tDCS during testing) 1.5 mA	Yes (on P300 amplitude and RT, not on subjective fatigue)	- P300 amplitude and latency during an auditory oddball task - simple RT in an alertness test	Crossover, sham-controlled (1-week wash-out)	15 (14 RR, 1 SP)	Clinically definite MS (121) with a minimum of 9 points on the cognitive subscale of the WEIMuS age: 43.20 ± 14.97 EDSS: 3.54 ± 1.94 WEIMuS physical: 19.73 ± 5.70	itching

(Continued)

TABLE 3 | Continued

References	Anode: location dimensions	Cathode: location dimensions	Stim duration stim intensity	Efficacy	Fatigue evaluation methods	Study design	Sample size and MS subtype	Sample composition (values expressed as mean ± SD, when available)	Adverse effects
					- subjective fatigue via a 10-point numerical rating scale and objective fatigue (e.g., WEIMuS physical)				
Ferrucci et al. (107)	Bilateral motor cortex 5 × 7 cm	Right deltoid 5 × 7 cm	15 min/day, 5 days 1.5 mA	Yes (n23, 15 responders)	FIS	Crossover, sham controlled (1-month wash-out)	25 (22 RR, 3 SP)	Clinically definite MS (121) Responders (n = 15): - age: 40.3 ± 2.3 - EDSS: 3 ± 0.4 - FIS anodal: 59.5 ± 7.1 - FIS sham: 49.8 ± 7 Non-responders (n = 8): - age: 52.5 ± 4.1 - EDSS: 3.8 ± 0.7 - FIS anodal: 58.5 ± 10.7 - FISsham: 61 ± 11.4	Skin reaction
Tecchio et al. (112)	Whole-body bilateral somatosensory cortex Custom-sized S1 electrode using individual brain MRI data 35 cm^2	Oz (EEG 10–20 system) 7 × 10 cm	15 min/day, 5 days 1.5 mA	Yes	MFIS	Crossover, sham-controlled (washout individually calculated by MFIS compared to baseline)	10 (7 RR, 1 SP, 2 PP)	MS in a mild state (EDSS < 3.5) with MFIS > 38 age: 45.8 ± 7.6 EDSS: 1.5 (range 0–3.5) MFIS: 41.6 ± 6.4	None reported
Tecchio et al. (111)		Oz (EEG 10–20 system) 6 × 14 cm	15 min/day, 5 days 1.5 mA	Yes	MFIS	Crossover, sham-controlled (washout individually calculated by MFIS compared to baseline	13 RR	MS patients with physical items mFIS score > 15 age: 45.8 ± 7.6 EDSS: 1.5 (range 0–3.5) MFIS: 41.6 ± 7.5	None reported
Cancelli et al. (109)		Oz (EEG 10–20 system) 7 × 10 cm	15 min/day, 5 days 1.5 mA	Yes	MFIS	Crossover, sham-controlled	10 RR	MS patients (121) with physical items mFIS score > 35 Age: 43.2 ± 13.1	None

(Continued)

TABLE 3 | Continued

References	Anode: location dimensions	Cathode: location dimensions	Stim duration stim intensity	Efficacy	Fatigue evaluation methods	Study design	Sample size and MS subtype	Sample composition (values expressed as mean ± SD, when available)	Adverse effects
								EDSS: 0.9 (range 0–3.5) MFIS: 46.6 ± 15.9	
Porcaro et al. (110)		Oz (EEG 10–20 system) 7 × 10 cm	15 min/day, 5 days 1.5 mA	Yes	MFIS	Crossover, sham controlled (washout individually calculated by MFIS compared to baseline	18 RR	MS patients with EDSS < 3.5 and mFIS score > 30 Age: 44.5 ± 10.4 EDSS: 1.1 (range 0–3.5) MFIS pre-real: 45.6 ± 31.66 MFIS pre sham: 44.9 ± 30.67	None reported
Tecchio et al. (111)	Bilateral sensorimotor hand area 70 m^2	Under the chin 84 cm^2	15 min/day, 5 days 1.5 mA	No	MFIS	Crossover, sham-controlled (washout individually calculated by MFIS compared to baseline)	8 RR	MS patients with physical items mFIS score > 15 age: 38.1 ± 9.8 EDSS: 2 (range 1–2.5) MFIS: 57.1 ± 19.9	None reported
Hanken et al. (108)	Right parietal cortex (P4) 5 × 7 cm	Right forehead 6 × 15 cm	Single session 20 min	Yes (RT) No (subjective fatigue) Only in subgroup with mild to moderate cognitive fatigue	- RT during a vigilance task - subjective fatigue (VAS)	Randomized, sham-controlled	46 (18 RR, 28 SP) analyzed 20 for each arm, divided in subgroups according to cognitive fatigue assessed by FSMC	MS patients (121) Mild/moderate CF active: - age: 51.8 ± 9.9 - EDSS: 4.0 ± 1.5 Severe CF active: - age: 50.9 ± 8.8 - EDSS: 4.8 ± 1.2 Mild/moderate CF sham: - age: 47.1 ± 10.3 - EDSS: 3.4 ± 2.1 Severe CF sham: - age: 46.5 ± 9.1 - EDSS: 4.5 ± 1.0	None reported

References	Stimulation location	TMS protocol TMS coil	Stimulation parameters	Efficacy	Fatigue evaluation methods	Study design	Sample size and MS subtype	Sample composition (values expressed as mean ± SD, when available)	Adverse effects
2) TMS									
Mori et al. (119)	M1 leg area contralateral to the affected limb	iTBS + individualized ET (2 h/day for 2 weeks)	1 session/day for 10 sessions over 2 weeks	Yes (real iTBS + exercise therapy group)	FSS Seconday outcome	Randomized, sham-controlled	20 RR	Definite RR MS (121) patients with spasticity affecting predominantly one lower limb Active	Treatment was generally well-tolerated.

(Continued)

TABLE 3 | Continued

References	Stimulation location	TMS protocol TMS coil	Stimulation parameters	Efficacy	Fatigue evaluation methods	Study design	Sample size and MS subtype	Sample composition (values expressed as mean ± SD, when available)	Adverse effects
			10 bursts of 3 stimuli at 50 Hz, repeated at 5 Hz every 10 s, for a total of 600 stimuli; biphasic waveform 80% AMT					iTBS + ET (n = 10): - age: 38.1 ± 10.7 - EDSS: 3.6 ± 1.2 - FSS: 39.5 ± 4.2 Sham iTBS + ET (n = 10): - age: 37.7 ± 12.3 - EDSS: 3.8 ± 1.6 Active iTBS only (n = 10): - age: 38.3 ± 11.9 - EDSS: 3.5 ± 1.0	
Gaede et al. (117)	a) left PFC (sham-controlled) b) bilateral M1	Deep TMS a) H6-coil b) H10-coil (bihemispherical stimulation)	18 sessions (3/week) over 6 weeks a) 50 bursts of 36 stimuli, 18 Hz, 120% RMT, ITI 20 s, 18 min b) 40 bursts of 20 stimuli, 5 Hz, ITI 20 s, 90% RMT, 16 min	Yes (more pronounced for bilateral M1)	FSS	Randomized, sham-controlled	9 PCF real, 10 PFC sham, 9 M1	MS diagnosis (121), with FSS > 4 or BDI-IA > 12 PFC real: - age: 47 (32–51) - EDSS: 2.5 (2.0–3.0) PFC sham: - age: 41 (39–45) - EDSS: 3.0 (2.5–3.0) M1 real: - age: 46 (42–48) - EDSS: 2.5 (2.5–3.5)	None serious: headache (30%), paresthesia or pain, gait disturbance, dizziness, tiredness, legs/bladder spasticity, discomfort
Korzhova et al. (118)	Bilateral M1	a) 20 Hz rTMS f8 coil b) iTBS + physical therapy (45–55 min/session)	1/day for 5 consecutive days, for 2 weeks a) 2 s on, 28 s off, 1,600 stimuli, 80% RMT, 30 min b) 10 bursts of 3 stimuli at 35 Hz, ITI 5 Hz, 1,200 stimuli/session, 80% RMT, 10 min	Yes (20 Hz rTMS group only)	MFIS Secondary outcome	Randomized, sham-controlled	34 SP (12 in the 20 Hz-rTMS group, 12 in the iTBS group, 10 in the sham group)	SP MS diagnosis according to McDonald criteria 2010 and lower spastic paraparesis with MAS > 2 measured in the knee - age: 45 (mean) - EDSS: 6.5 (mean)	None reported

(Continued)

TABLE 3 | Continued

References	Anode: location dimensions	Cathode: location dimensions	Stim duration stim intensity	Efficacy	Fatigue evaluation methods	Study design	Sample size and MS subtype	Sample composition (values expressed as mean ± SD, when available)	Adverse effects
3) tRNS									
Palm et al. (113)	F3 (EEG 10–20 system) 25 cm^2	AF8 (EEG 10–20 system) 25 cm^2	20 min/day for 3 days Peak to peak amplitude of 2 mA, full-band white noise from 0 to 500 Hz, variance 650/2 μA	No	MFIS	Crossover, sham-controlled (3-weeks wash-out)	16 (11 RR, 4 SP, 1 PP)	Clinically definite MS (121) and history of neuropathic pain with VAS > 40 age: 47.4 ± 8.9 EDSS: 4.2 ± 1.3 MFIS: 52.6 ± 12.3	Phosphenes, insomnia, nausea, severe headache (1, after sham)

DLPFC, dorsolateral prefrontal cortex; EEG, electroencephalography; FSS, Fatigue Severity Scale; iTBS, intermittent theta burst stimulation; ITI, inter-train interval; MFIS, Modified Fatigue Impact Scale; MSFSS, MS-specific FSS; MRI, magnetic resonance imaging; PFC, prefrontal cortex; PP, primary progressive; PPC, posterior parietal cortex; PROMIS, Patient-Reported Outcomes Measurement Information System; RMT, resting motor threshold; RR, relapsing–remitting; RT,reaction time; SP, secondary progressive; rTMS, repetitive transcranial magnetic stimulation; tDCS, transcranial direct current stimulation; tRNS, transcranial random noise stimulation; VAS, visual analog scale for fatigue.

MRI-guided neuronavigation system. In a more recent study of this group, the importance of the individual baseline neural networks activity has been outlined as a further parameter for individualized treatment (110). The results of their studies support the efficacy of personalized tDCS approaches.

Only one sham-controlled study has explored the effects on MS-related fatigue of another NIBS technique called transcranial random-noise stimulation (tRNS). This stimulation was applied on frontal regions but produced negative results (113).

The other NIBS technique introduced in the research setting for the treatment of fatigue in PwMS is TMS. (114). Repetitive protocols of TMS showed long-lasting effects on cortical excitability in patients with stroke (115), MS-related spasticity (116) and major depression (13).

Regarding MS-related fatigue, three sham-controlled studies using TMS showed promising results (117–119). Two of these studies used TMS in combination with physical therapy and enrolled patients affected by spasticity (118, 119). Different TMS protocols were used: intermittent theta-burst stimulation (iTBS) applied to the M1 leg area (119), deep TMS, delivered with specific H-coils to the left prefrontal cortex and to bilateral M1 (117), and 20-Hz repetitive TMS and iTBS applied to bilateral M1 (118). Preliminary evidence of efficacy was described for all the protocols excepted for iTBS on bilateral M1 (118).

In a recent systematic meta-analysis, Liu et al. reviewed the efficacy and safety of NIBS specifically for the treatment of MS-related fatigue (120). They performed a literature search for sham-controlled brain stimulation studies based on tDCS, rTMS, tRNS, and transcranial alternating current stimulation (tACS). A total of 14 eligible studies published from 2011 to 2018, for a total of 207 MS patients, were found: 11 tDCS studies, one rTMS study, one iTBS (combined with exercise therapy) study, and one tRNS study. A significant improvement in fatigue scores compared to sham was found after tDCS treatment. A subgroup analysis demonstrated significance for the intensity of 1.5 mA and for bilateral S1 stimulation location. The two TMS studies and the tRNS study did not reach statistical significance.

Several data are available about the therapeutic use of NIBS for reducing MS-related fatigue (**Table 3**). These techniques—and in particular tDCS and some TMS protocols—have shown to be effective as add-on therapy for fatigue management, and more studies are needed to explore their further implementation. The mechanisms by which NIBS could improve fatigue are still unclear (8, 15, 104). Different hypothesis have been proposed such as presynaptic increase of spinal drive from motor cortex, modulation of premotor areas, increase in motivation, decrease in muscle pain, increase in muscle coupling, promotion of changes in cortical resting state activity and cortico-cortical connectivity, and induction of long-term potentiation-like and long-term depression-like neuroplastic changes at a local and/or network level. The potential role of altered oscillatory activity in the pathogenesis of MS-related cognitive fatigue and the potential advantage of tACS application have also been outlined (122). A better comprehension of the pathogenesis could be useful to develop therapies that specifically target the mechanisms of fatigue generation in MS.

The studies published so far are greatly heterogeneous, differing in many variables such as the NIBS technique used, the

cortical targets, the stimulation intensity, and the characteristics of the populations included. Indeed although most of the studies enrolled patients with EDSS ≤ 6, other population characteristics were more heterogeneous among studies, such as the MS subtype, the presence of comorbidities, the measured outcome in addition to spasticity (e.g., neuropathic pain), and the baseline fatigue scores.

Other important limitations to the use of NIBS for therapeutic purpose remain the still heterogeneous definition of fatigue, the limited comprehension of its complex and multifactorial pathophysiology, and the limited use of objective measures other than self-report questionnaires.

Because of this methodological heterogeneity and the low sample sizes, the level of evidence for NIBS efficacy resulted too low to draw any robust conclusion to support its use in clinical practice (15) but encourages further studies on NIBS as a treatment for fatigue (120).

CONCLUSIONS

Several studies have used neurophysiological tools to evaluate MS-related fatigue. Until now, this possibility has been mainly exploited for investigating the pathogenic mechanisms of fatigue and for modulating brain circuits for therapeutic purposes. The potential role of neurophysiology for quantifying fatigue and predicting and/or monitoring response to treatment has been evaluated in only a few studies.

From a methodological perspective, the most used techniques are TMS and tDCS. TMS is a very versatile method that allows both to assess, non-invasively, the functionality of corticospinal tract and cortico-cortical connections and, when delivered in repetitive protocols, to modulate brain activity (114). On the other side, tDCS is the most investigated technique as a potential treatment for fatigue because it is safe, well-tolerated, low-cost, and portable (13, 15).

Other neurophysiological techniques have been used, although in a relatively small number of studies. In particular, EEG has been used for exploring the role of cortico-cortical connections (34), EMG for evaluating the contribution of peripheral structures (9), evoked potentials for investigating the pathogenetic mechanisms (60) and predicting response to pharmacological treatment (94) and autonomic nervous system testing and polysomnography for assessing additional factors that can produce or exacerbate fatigue in PwMS.

Most part of the studies have been conducted in small samples by comparing the findings obtained in fatigued MS patients with those obtained in healthy controls or not-fatigued MS patients. Usually, each study used a single neurophysiological technique, while few studies combined different neurophysiological techniques (83) or neurophysiology with MRI (58).

Overall the literature data presented in this review demonstrate that neurophysiology could play a role in the management and evaluation of MS-related fatigue. Despite of heterogeneity in results and methodological limitations, current evidence supports further studies on the role of neurophysiology in the management of fatigue. In particular, for therapeutic purpose, tailored approaches based on individual network dysfunctions, individual plasticity impairment, and other neurophysiological variables should be explored.

AUTHOR CONTRIBUTIONS

FC, FM, EF, and MR wrote the manuscript. FC, FM, and VD critically revised the manuscript.

REFERENCES

1. Induruwa I, Constantinescu CS, Gran B. Fatigue in multiple sclerosis — a brief review. *J Neurol Sci*. (2012) 323:9–15. doi: 10.1016/j.jns.2012.08.007
2. Ayache SS, Chalah MA. Fatigue in multiple sclerosis - insights into evaluation and management. *Neurophysiol Clin*. (2017) 47:139–71. doi: 10.1016/j.neucli.2017.02.004
3. Yang TT, Wang L, Deng XY, Yu G. Pharmacological treatments for fatigue in patients with multiple sclerosis: a systematic review and meta-analysis. *J Neurol Sci*. (2017) 380:256–61. doi: 10.1016/j.jns.2017.07.042
4. Rottoli M, La Gioia S, Frigeni B, Barcella V. Pathophysiology, assessment and management of multiple sclerosis fatigue: an update. *Expert Rev Neurother*. (2017) 17:373–9. doi: 10.1080/14737175.2017.1247695
5. Taylor JL, Gandevia SC. A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. *J Appl Physiol.* (2008) 104:542–50. doi: 10.1152/japplphysiol.01053.2007
6. Capone F, Collorone S, Cortese R, Di Lazzaro V, Moccia M. Fatigue in multiple sclerosis: the role of thalamus. *Mult Scler*. (2020) 26:6–16. doi: 10.1177/1352458519851247
7. Chaudhuri A, Behan PO. Fatigue in neurological disorders. *Lancet*. (2004) 363:978–88. doi: 10.1016/S0140-6736(04)15794-2
8. Chalah MA, Riachi N, Ahdab R, Créange A, Lefaucheur JP, Ayache SS. Fatigue in multiple sclerosis: neural correlates and the role of non-invasive brain stimulation. *Front Cell Neurosci.* (2015) 9:460. doi: 10.3389/fncel.2015.00460
9. Zwarts MJ, Bleijenberg G, van Engelen BG. Clinical neurophysiology of fatigue. *Clin Neurophysiol.* (2008) 119:2–10. doi: 10.1016/j.clinph.2007.09.126
10. Leocani L, Comi G. Neurophysiological investigations in multiple sclerosis. *Curr Opin Neurol*. (2000) 13:255–61. doi: 10.1097/00019052-200006000-00004
11. Woods AJ, Antal A, Bikson M, Boggio PS, Brunoni AR, Celnik P, et al. A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clin Neurophysiol*. (2016) 127:1031–48. doi: 10.1016/j.clinph.2015.11.012
12. Perera T, George MS, Grammer G, Janicak PG, Pascual-Leone A, Wirecki TS. The clinical TMS society consensus review and treatment recommendations for TMS therapy for major depressive disorder. *Brain Stimul*. (2016) 9:336–46. doi: 10.1016/j.brs.2016.03.010
13. Lefaucheur JP, Aleman A, Baeken C, Benninger DH, Brunelin J, Di Lazzaro V, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): an update (2014-2018). *Clin Neurophysiol*. (2020) 131:474–528. doi: 10.1016/j.clinph.2019.11.002
14. Iodice R, Manganelli F, Dubbioso R. The therapeutic use of non-invasive brain stimulation in multiple sclerosis - a review. *Restor Neurol Neurosci*. (2017) 35:497–509. doi: 10.3233/RNN-170735
15. Lefaucheur JP, Chalah MA, Mhalla A, Palm U, Ayache SS, Mylius V. The treatment of fatigue by non-invasive brain stimulation. *Neurophysiol Clin*. (2017) 47:173–84. doi: 10.1016/j.neucli.2017.03.003

16. Bigland-Ritchie B, Woods JJ. Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve*. (1984) 7:691–9. doi: 10.1002/mus.880070902
17. Rice CL, Vollmer TL, Bigland-Ritchie B. Neuromuscular responses of patients with multiple sclerosis. *Muscle Nerve*. (1992) 15:1123–32. doi: 10.1002/mus.880151011
18. Sheean GL, Murray NM, Rothwell JC, Miller DH, Thompson AJ. An electrophysiological study of the mechanism of fatigue in multiple sclerosis. *Brain*. (1997) 120:299–315. doi: 10.1093/brain/120.2.299
19. Perretti A, Balbi P, Orefice G, Trojano L, Marcantonio L, Brescia-Morra V, et al. Post-exercise facilitation and depression of motor evoked potentials to transcranial magnetic stimulation: a study in multiple sclerosis. *Clin Neurophysiol*. (2004) 115:2128–33. doi: 10.1016/j.clinph.2004.03.028
20. Jørgensen M, Dalgas U, Wens I, Hvid LG. Muscle strength and power in persons with multiple sclerosis - a systematic review and meta-analysis. *J Neurol Sci*. (2017) 376:225–41. doi: 10.1016/j.jns.2017.03.022
21. Steens A, de Vries A, Hemmen J, Heersema T, Heerings M, Maurits N, et al. Fatigue perceived by multiple sclerosis patients is associated with muscle fatigue. *Neurorehabil Neural Repair*. (2012) 26:48–57. doi: 10.1177/1545968311416991
22. Steens A, Heersema DJ, Maurits NM, Renken RJ, Zijdewind I. Mechanisms underlying muscle fatigue differ between multiple sclerosis patients and controls: a combined electrophysiological and neuroimaging study. *Neuroimage*. (2012) 59:3110–8. doi: 10.1016/j.neuroimage.2011.11.038
23. Wolkorte R, Heersema DJ, Zijdewind I. Reduced voluntary activation during brief and sustained contractions of a hand muscle in secondary-progressive multiple sclerosis patients. *Neurorehabil Neural Repair*. (2016) 30:307–16. doi: 10.1177/1545968315593809
24. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol*. (2000) 10:361–74. doi: 10.1016/S1050-6411(00)00027-4
25. Drost G, Stegeman DF, van Engelen BG, Zwarts MJ. Clinical applications of high-density surface EMG: a systematic review. *J Electromyogr Kinesiol*. (2006) 16:586–602. doi: 10.1016/j.jelekin.2006.09.005
26. Kandel ER, Schwartz JH. (editors). *Principles of Neural Science*. 4th ed. New York, NY: McGraw-Hill (2000).
27. Garland SJ, Enoka RM, Serrano LP, Robinson GA. Behavior of motor units in human biceps brachii during a submaximal fatiguing contraction. *J Appl Physiol*. (1985) 76:2411–9. doi: 10.1152/jappl.1994.76.6.2411
28. Merletti R, Knaflitz M, De Luca CJ. Myoelectric manifestations of fatigue in voluntary and electrically elicited contractions. *J Appl Physiol*. (1985) 69:1810–20. doi: 10.1152/jappl.1990.69.5.1810
29. Behm DG. Force maintenance with submaximal fatiguing contractions. *Can J Appl Physiol*. (2004) 29:274–90. doi: 10.1139/h04-019
30. De Luca CJ. Myoelectrical manifestations of localized muscular fatigue in humans. *Crit Rev Biomed Eng*. (1984) 11:251–79.
31. Kallenberg LA, Hermens HJ. Behaviour of a surface EMG based measure for motor control: motor unit action potential rate in relation to force and muscle fatigue. *J Electromyogr Kinesiol*. (2008) 18:780–8. doi: 10.1016/j.jelekin.2007.02.011
32. Eken MM, Richards R, Beckerman H, van der Krogt M, Gerrits K, Rietberg M, et al. Quantifying muscle fatigue during walking in people with multiple sclerosis. *Clin Biomech*. (2019) 72:94–101. doi: 10.1016/j.clinbiomech.2019.11.020
33. Severijns D, Octavia JR, Kerkhofs L, Coninx K, Lamers I, Feys P. Investigation of fatigability during repetitive robot-mediated arm training in people with multiple sclerosis. *PLoS ONE*. (2015) 10:e0133729. doi: 10.1371/journal.pone.0133729
34. Leocani L, Colombo B, Magnani G, Martinelli-Boneschi F, Cursi M, Rossi P, et al. Fatigue in multiple sclerosis is associated with abnormal cortical activation to voluntary movement–EEG evidence. *Neuroimage*. (2001) 13(Pt 1):1186–92. doi: 10.1006/nimg.2001.0759
35. Gandevia SC, Allen GM, Butler JE, Taylor JL. Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J Physiol*. (1996) 490:529–36. doi: 10.1113/jphysiol.1996.sp021164
36. Petajan JH, White AT. Motor-evoked potentials in response to fatiguing grip exercise in multiple sclerosis patients. *Clin Neurophysiol*. (2000) 111:2188–95. doi: 10.1016/S1388-2457(00)00469-7
37. Liepert J, Mingers D, Heesen C, Baumer T, Weiller C. Motor cortex excitability and fatigue in multiple sclerosis: a transcranial magnetic stimulation study. *Mult Scler*. (2005) 11:316–21. doi: 10.1191/1352458505ms1163oa
38. Thickbroom GW, Sacco P, Faulkner DL, Kermode AG, Mastaglia FL. Enhanced corticomotor excitability with dynamic fatiguing exercise of the lower limb in multiple sclerosis. *J Neurol*. (2008) 255:1001–5. doi: 10.1007/s00415-008-0818-6
39. Mordillo-Mateos L, Soto-Leon V, Torres-Pareja M, Peinado-Palomino D, Mendoza-Laiz N, Alonso-Bonilla C, et al. Fatigue in multiple sclerosis: general and perceived fatigue does not depend on corticospinal tract dysfunction. *Front Neurol*. (2019) 10:339. doi: 10.3389/fneur.2019.00339
40. Morgante F, Dattola V, Crupi D, Russo M, Rizzo V, Ghilardi MF, et al. Is central fatigue in multiple sclerosis a disorder of movement preparation? *J Neurol*. (2011) 258:263–72. doi: 10.1007/s00415-010-5742-x
41. Russo M, Crupi D, Naro A, Avanzino L, Buccafusca M, Dattola V, et al. Fatigue in patients with multiple sclerosis: from movement preparation to motor execution. *J Neurol Sci*. (2015) 351:52–7. doi: 10.1016/j.jns.2015.02.031
42. Di Lazzaro V, Rothwell J, Capogna M. Noninvasive Stimulation of the Human Brain: Activation of Multiple Cortical Circuits. *Neuroscientist*. (2018) 24:246–60. doi: 10.1177/1073858417717660
43. Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. *J Physiol*. (1993) 471:501–19. doi: 10.1113/jphysiol.1993.sp019912
44. Di Lazzaro V, Oliviero A, Meglio M, Cioni B, Tamburrini G, Tonali P, et al. Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. *Clin Neurophysiol*. (2000) 111:794–9. doi: 10.1016/S1388-2457(99)00314-4
45. Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. *J Physiol*. (1996) 496:873–81. doi: 10.1113/jphysiol.1996.sp021734
46. Di Lazzaro V, Pilato F, Oliviero A, Dileone M, Saturno E, Mazzone P, et al. Origin of facilitation of motor-evoked potentials after paired magnetic stimulation: direct recording of epidural activity in conscious humans. *J Neurophysiol*. (2006) 96:1765–71. doi: 10.1152/jn.00360.2006
47. Valls-Solé J, Pascual-Leone A, Wassermann EM, Hallett M. Human motor evoked responses to paired transcranial magnetic stimuli. *Electroencephalogr Clin Neurophysiol*. (1992) 85:355–64.
48. McDonnell MN, Orekhov Y, Ziemann U. The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Exp Brain Res*. (2006) 173:86–93. doi: 10.1007/s00221-006-0365-2
49. Benwell NM, Sacco P, Hammond GR, Byrnes ML, Mastaglia FL, Thickbroom GW. Short-interval cortical inhibition and corticomotor excitability with fatiguing hand exercise: a central adaptation to fatigue? *Exp Brain Res*. (2006) 170:191–8. doi: 10.1007/s00221-005-0195-7
50. Maruyama A, Matsunaga K, Tanaka N, Rothwell JC. Muscle fatigue decreases short-interval intracortical inhibition after exhaustive intermittent tasks. *Clin Neurophysiol*. (2006) 117:864–70. doi: 10.1016/j.clinph.2005.12.019
51. Hunter SK, McNeil CJ, Butler JE, Gandevia SC, Taylor JL. Short-interval cortical inhibition and intracortical facilitation during submaximal voluntary contractions changes with fatigue. *Exp Brain Res*. (2016) 234:2541–51 doi: 10.1007/s00221-016-4658-9
52. Cinelli K, Green LA, Kalmar JM. The task at hand: fatigue-associated changes in cortical excitability during writing. *Brain Sciences*. (2019) 9:353. doi: 10.3390/brainsci9120353
53. Chalah MA, Kauv P, Créange A, Hodel J, Lefaucheur JP, Ayache SS. Neurophysiological, radiological and neuropsychological evaluation of fatigue in multiple sclerosis. *Mult Scler Relat Disord*. (2019) 28:145–52. doi: 10.1016/j.msard.2018.12.029
54. Epstein C, Wassermann E, Ziemann U. *Oxford Handbook of Transcranial Stimulation*. Oxford: Oxford University Press (2012).
55. Chaves AR, Kelly LP, Moore CS, Stefanelli M, Ploughman M. Prolonged cortical silent period is related to poor fitness and fatigue, but not tumor

necrosis factor, in multiple sclerosis. *Clin Neurophysiol*. (2019) 130:474–83. doi: 10.1016/j.clinph.2018.12.015
56. Leocani L, Chieffo R, Gentile A, Centonze D. Beyond rehabilitation in MS: insights from non-invasive brain stimulation. *Mult Scler*. (2019) 25:1363–71. doi: 10.1177/1352458519865734
57. Stampanoni Bassi M, Buttari F, Maffei P, De Paolis N, Sancesario A, Gilio L, et al. Practice-dependent motor cortex plasticity is reduced in non-disabled multiple sclerosis patients. *Clin Neurophysiol*. (2020) 131:566–73. doi: 10.1016/j.clinph.2019.10.023
58. Conte A, Li Voti P, Pontecorvo S, Quartuccio ME, Baione V, Rocchi L, et al. Attention-related changes in short-term cortical plasticity help to explain fatigue in multiple sclerosis. *Mult Scler*. (2016) 22:1359–56. doi: 10.1177/1352458515619780
59. Classen J, Wolters A, Stefan K, Wycislo M, Sandbrink F, Schmidt A, et al. Paired associative stimulation. *Suppl Clin Neurophysiol*. (2004) 57:563–9. doi: 10.1016/S1567-424X(09)70395-2
60. Capone F, Motolese F, Rossi M, Musumeci G, Insola A, Di Lazzaro V. Thalamo-cortical dysfunction contributes to fatigability in multiple sclerosis patients and neurophysiological study. *Mult Scler Relat Disord.* (2019) 39:101897. doi: 10.1016/j.msard.2019.101897
61. Chinnadurai SA, Venkatesan SA, Shankar G, Samivel B, Ranganathan LN. A study of cognitive fatigue in multiple sclerosis with novel clinical and electrophysiological parameters utilizing the event related potential P300. *Mult Scler Relat Disord*. (2016) 10:1–6. doi: 10.1016/j.msard.2016.08.001
62. Lazarevic S, Azanjac Arsic A, Aleksic D, Toncev G, Miletic-Drakulic S. Depression and fatigue in patients with multiple sclerosis have no influence on the parameters of cognitive evoked potentials. *J Clin Neurophysiol*. (2019). doi: 10.1097/WNP.0000000000000640. [Epub ahead of print].
63. Braley TJ, Segal BM, Chervin RD. Sleep-disordered breathing in multiple sclerosis. *Neurology*. (2012) 79:929–36. doi: 10.1212/WNL.0b013e318266fa9d
64. Deriu M, Cossu G, Molari A, Murgia D, Mereu A, Ferrigno P, et al. Restless legs syndrome in multiple sclerosis: a case–control study. *Mov Disord.* (2009) 24:697–701. doi: 10.1002/mds.22431
65. Manconi M, Ferini-Strambi L, Filippi M, Bonanni E, Iudice A, Murri L, et al. Multicenter case-control study on restless legs syndrome in multiple sclerosis: the REMS study. *Sleep*. (2008) 31:944–52.
66. Ferini-Strambi L, Filippi M, Martinelli V, Oldani A, Rovaris M, Zucconi M, et al. Nocturnal sleep study in multiple sclerosis: correlations with clinical and brain magnetic resonance imaging findings. *J Neurol Sci*. (1994) 125:194–7. doi: 10.1016/0022-510X(94)90035-3
67. Gómez-Choco MJ, Iranzo A, Blanco Y, Graus F, Santamaría J, Saiz A. Prevalence of restless legs syndrome, and, REM sleep behavior disorder in multiple sclerosis. *Mult Scler*. (2007) 13:805–8. doi: 10.1177/1352458506074644
68. Chinnadurai SA, Gandhirajan D, Pamidimukala V, Kesavamurthy B, Venkatesan SA. Analysing the relationship between polysomnographic measures of sleep with measures of physical and cognitive fatigue in people with multiple sclerosis. *Mult Scler Relat Disord.* (2018) 24:32–7. doi: 10.1016/j.msard.2018.05.016
69. Habek M, Adamec I, Barun B, Crnošija L, Gabelić T, Krbot Skorić M. Clinical neurophysiology of multiple sclerosis. *Adv Exp Med Biol.* (2017) 958:129–39. doi: 10.1007/978-3-319-47861-6_8
70. Bridoux A, Créange A, Sangare A, Ayache SS, Hosseini H, Drouot X, et al. Impaired sleep-associated modulation of post-exercise corticomotor depression in multiple sclerosis. *J Neurol Sci*. (2015) 354:91–6. doi: 10.1016/j.jns.2015.05.006
71. Low PA, Caskey PE, Tuck RR, Fealey RD, Dyck PJ. Quantitative sudomotor axon reflex test in normal and neuropathic subjects. *Ann Neurol*. (1983) 14:573–80. doi: 10.1002/ana.410140513
72. Freeman JV, Dewey FE, Hadley DM, Myers J, Froelicher VF. Autonomic nervous system interaction with the cardiovascular system during exercise. *Prog Cardiovasc Dis*. (2006) 48:342–62. doi: 10.1016/j.pcad.2005.11.003
73. Findling O, Hauer L, Pezawas T, Rommer PS, Struhal W, Sellner J. Cardiac autonomic dysfunction in multiple sclerosis: a systematic review of current knowledge and impact of immunotherapies. *J Clin Med*. (2020) 24:9. doi: 10.3390/jcm9020335
74. Lebre AT, Mendes MF, Tilbery CP, Almeida AL, Scatolini Neto A. Relation between fatigue and autonomic disturbances in multiple sclerosis. *Arq Neuro Psiquiatr.* (2007) 65:663–8. doi: 10.1590/S0004-282X2007000400023
75. Flachenecker P, Rufer A, Bihler I, Hippel C, Reiners K, Toyka K, et al. Fatigue in MS is related to sympathetic vasomotor dysfunction. *Neurology*. (2003) 61:851–3. doi: 10.1212/01.WNL.0000080365.95436.B8
76. Niepel G, Bibani RH, Vilisaar J, Langley RW, Bradshaw CM, Szabadi E, et al. Association of a deficit of arousal with fatigue in multiple sclerosis: effect of modafinil. *Neuropharmacology*. (2013) 64:380–8. doi: 10.1016/j.neuropharm.2012.06.036
77. Keselbrener L, Akselrod S, Ahiron A, Eldar M, Barak Y, Rotstein Z. Is fatigue in patients with multiple sclerosis related to autonomic dysfunction? *Clin Auton Res*. (2000) 10:169–75. doi: 10.1007/BF02291352
78. Heesen C, Koehler G, Gross R, Tessmer W, Schulz KH, Gold SM. Altered cytokine responses to cognitive stress in multiple sclerosis patients with fatigue. *Mult Scler*. (2005) 11:51–7. doi: 10.1191/1352458505ms1129oa
79. Sander C, Modes F, Schlake H-P, Eling P, Hildebrandt H. Capturing fatigue parameters: The impact of vagal processing in multiple sclerosis related cognitive fatigue. *Mult Scler Relat Disord*. (2019) 32:13–8. doi: 10.1016/j.msard.2019.04.013
80. de Rodez Benavent SA, Nygaard GO, Harbo HF, Tønnesen S, Sowa P, Landrø, NI, et al. Fatigue and cognition: pupillary responses to problem-solving in early multiple sclerosis patients. *Brain Behav*. (2017) 7:e00717doi: 10.1002/brb3.717
81. Rocca MA, Parisi L, Pagani E, Copetti M, Rodegher M, Colombo B, et al. Regional but not global brain damage contributes to fatigue in multiple sclerosis. *Radiology*. (2014) 273:511–20. doi: 10.1148/radiol.14140417
82. Hidalgo de la Cruz M, d'Ambrosio A, Valsasina P, Pagani E, Colombo B, Rodegher M, et al. Abnormal functional connectivity of thalamic sub-regions contributes to fatigue in multiple sclerosis. *Mult Scler*. (2018) 24:1183–95. doi: 10.1177/1352458517717807
83. Sandroni P, Walker C, Starr A. 'Fatigue' in Patients With Multiple Sclerosis: Motor Pathway Conduction and Event-Related Potentials. *Arch Neurol*. (1992) 49:517–24. doi: 10.1001/archneur.1992.00530290105019
84. Russo M, Calamuneri A, Cacciola A, Bonanno L, Naro A, Dattola V, et al. Neural correlates of fatigue in multiple sclerosis: a combined neurophysiological and neuroimaging approach (R1). *Arch Ital Biol.* (2017) 155:142–51. doi: 10.12871/00039829201735
85. Pokryszko-Dragan A, Zagrajek M, Slotwinski K, Bilinska M, Gruszka E, Podemski R. Event-related potentials and cognitive performance in multiple sclerosis patients with fatigue. *Neurol Sci*. (2016) 37:1545–56. doi: 10.1007/s10072-016-2622-x
86. Pokryszko-Dragan A, Dziadkowiak E, Zagrajek M, Slotwinski K, Gruszka E, Bilinska M, et al. Cognitive performance, fatigue and event-related potentials in patients with clinically isolated syndrome. *Clin Neurol Neurosurg.* (2016) 149:68–74. doi: 10.1016/j.clineuro.2016.07.022
87. Brenner P, Piehl F. Fatigue and depression in multiple sclerosis: pharmacological and non-pharmacological interventions. *Acta Neurol Scand.* (2016) 134:47–54. doi: 10.1111/ane.12648
88. Scatton B, Cheramy A, Besson MJ, Glowinski J. Increased synthesis and release of dopamine in the striatum of the rat after amantadine treatment. *Eur J Pharmacol*. (1970) 13:131–3. doi: 10.1016/0014-2999(70)90194-9
89. Rosenberg GA, Appenzeller O. Amantadine, fatigue, and multiple sclerosis. *Arch Neurol*. (1988) 45:1104–6. doi: 10.1001/archneur.1988.00520340058012
90. Dobryakova E, Genova HM, DeLuca J, Wylie GR. The dopamine imbalance hypothesis of fatigue in multiple sclerosis and other neurological disorders. *Front Neurol.* (2015) 6:52. doi: 10.3389/fneur.2015.00052
91. Santarnecchi E, Rossi S, Bartalini S, Cincotta M, Giovannelli F, Tatti E, et al. Neurophysiological correlates of central fatigue in healthy, subjects and multiple sclerosis patients before and after treatment with amantadine. *Neural Plast.* (2015) 2015:616242. doi: 10.1155/2015/616242
92. Reis J, John D, Heimeroth A, Mueller HH, Oertel WH, Arndt T, et al. Modulation of human motor cortex excitability by single doses of amantadine. *Neuropsychopharmacology*. (2006) 31:2758–66. doi: 10.1038/sj.npp.1301122
93. Lange R, Volkmer M, Heesen C, Liepert J. Modafinil effects in multiple sclerosis patients with fatigue. *J Neurol*. (2009) 256:645–50. doi: 10.1007/s00415-009-0152-7

94. Nagels G, D'hooghe MB, Vleugels L, Kos D, Despontin M, De Deyn PP. P300 and treatment effect of modafinil on fatigue in multiple sclerosis. *J Clin Neurosci*. (2007) 14:33–40. doi: 10.1016/j.jocn.2005.10.008
95. Targ EF, Kocsis JD. 4-Aminopyridine leads to restoration of conduction in demyelinated rat sciatic nerve. *Brain Res*. (1985) 328:358–61. doi: 10.1016/0006-8993(85)91049-2
96. Mainero C, Inghilleri M, Pantano P, Conte A, Lenzi D, Frasca V, et al. Enhanced brain motor activity in patients with MS after a single dose of 3,4-diaminopyridine. *Neurology*. (2004) 62:2044–50. doi: 10.1212/01.WNL.0000129263.14219.A8
97. Sheean GL, Murray NM, Rothwell JC, Miller DH, Thompson AJ. An open-labelled clinical and electrophysiological study of 3,4 diaminopyridine in the treatment of fatigue in multiple sclerosis. *Brain*. (1998) 121:967–75. doi: 10.1093/brain/121.5.967
98. Marion S, Leonid C, Belinda B, Joanne D, Elise H, Leeanne C, et al. Effects of modified-release fampridine on upper limb impairment in patients with multiple sclerosis. *Mult Scler Relat Disord*. (2020) 40:101971. doi: 10.1016/j.msard.2020.101971
99. Cogliati Dezza I, Zito G, Tomasevic L, Filippi MM, Ghazaryan A, Porcaro C, et al. Functional and structural balances of homologous sensorimotor regions in multiple sclerosis fatigue. *J Neurol*. (2015) 262:614–22. doi: 10.1007/s00415-014-7590-6
100. Abdelmoula A, Baudry S, Duchateau J. Anodal transcranial direct current stimulation enhances time to task failure of a submaximal contraction of elbow flexors without changing corticospinal excitability. *Neuroscience*. (2016) 322:94–103. doi: 10.1016/j.neuroscience.2016.02.025
101. Cogiamanian F, Marceglia S, Ardolino G, Barbieri S, Priori A. Improved isometric force endurance after transcranial direct current stimulation over the human motor cortical areas. *Eur J Neurosci*. (2007) 26:242–9. doi: 10.1111/j.1460-9568.2007.05633.x
102. Saiote C, Goldschmidt T, Timaus C, Steenwijk MD, Opitz A, Antal A, et al. Impact of transcranial direct current stimulation on fatigue in multiple sclerosis. *Restor Neurol Neurosci*. (2014) 32:423–36. doi: 10.3233/RNN-130372
103. Ayache SS, Palm U, Chalah MA, Al-Ani T, Brignol A, Abdellaoui M, et al. Prefrontal tDCS decreases pain in patients with multiple sclerosis. *Front Neurosci*. (2016) 10:147. doi: 10.3389/fnins.2016.00147
104. Chalah MA, Riachi N, Ahdab R, Mhalla A, Abdellaoui M, Creange A, et al. Effects of left DLPFC versus right PPC tDCS on multiple sclerosis fatigue. *J Neurol Sci*. (2017) 372:131–7. doi: 10.1016/j.jns.2016.11.015
105. Charvet LE, Dobbs B, Shaw MT, Bikson M, Datta A, Krupp LB. Remotely supervised transcranial direct current stimulation for the treatment of fatigue in multiple sclerosis: results from a randomized, sham-controlled trial. *Mult Scler*. (2018) 24:1760–9. doi: 10.1177/1352458517732842
106. Fiene M, Rufener KS, Kuehne M, Matzke M, Heinze HJ, Zaehle T. Electrophysiological and behavioral effects of frontal transcranial direct current stimulation on cognitive fatigue in multiple sclerosis. *J Neurol*. (2018) 265:607–17. doi: 10.1007/s00415-018-8754-6
107. Ferrucci R, Vergari M, Cogiamanian F, Bocci T, Ciocca M, Tomasini E, et al. Transcranial direct current stimulation (tDCS) for fatigue in multiple sclerosis. *NeuroRehabil*. (2014) 34:121–7. doi: 10.3233/NRE-131019
108. Hanken K, Bosse M, Mohrke K, Eling P, Kastrup A, Antal A, et al. Counteracting fatigue in multiple sclerosis with right parietal anodal transcranial direct current stimulation. *Front Neurol*. (2016) 7:154. doi: 10.3389/fneur.2016.00154
109. Cancelli A, Cottone C, Giordani A, Migliore S, Lupoi D, Porcaro C, et al. Personalized, bilateral whole-body somatosensory cortex stimulation to relieve fatigue in multiple sclerosis. *Mult Scler*. (2018) 24:1366–74. doi: 10.1177/1352458517720528
110. Porcaro C, Cottone C, Cancelli A, Rossini PM, Zito G, Tecchio F. Cortical neurodynamics changes mediate the efficacy of a personalized neuromodulation against multiple sclerosis fatigue. *Sci Rep*. (2019) 9:18213. doi: 10.1038/s41598-019-54595-z
111. Tecchio F, Cancelli A, Cottone C, Ferrucci R, Vergari M, Zito G, et al. Brain plasticity effects of neuromodulation against multiple sclerosis fatigue. *Front Neurol*. (2015) 6:141. doi: 10.3389/fneur.2015.00141
112. Tecchio F, Cancelli A, Cottone C, Zito G, Pasqualetti P, Ghazaryan A, et al. Multiple sclerosis fatigue relief by bilateral somatosensory cortex neuromodulation. *J Neurol*. (2014) 261:1552–8. doi: 10.1007/s00415-014-7377-9
113. Palm U, Chalah MA, Padberg F, Al-Ani T, Abdellaoui M, Sorel M, et al. Effects of transcranial random noise stimulation (tRNS) on affect, pain and attention in multiple sclerosis. *Restor Neurol Neurosci*. (2016) 34:189–99. doi: 10.3233/RNN-150557
114. Hallett M. Transcranial magnetic stimulation: a primer. *Neuron*. (2007) 55:187–99. doi: 10.1016/j.neuron.2007.06.026
115. Hummel FC, Cohen LG. Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? *Lancet Neurol*. (2006) 5:708–12. doi: 10.1016/S1474-4422(06)70525-7
116. Centonze D, Koch G, Versace V, Mori F, Rossi S, Brusa L, et al. Repetitive transcranial magnetic stimulation of the motor cortex ameliorates spasticity in multiple sclerosis. *Neurology*. (2007) 68:1045–50. doi: 10.1212/01.wnl.0000257818.16952.62
117. Gaede G, Tiede M, Lorenz I, Brandt AU, Pfueller C, Dorr J, et al. Safety and preliminary efficacy of deep transcranial magnetic stimulation in MS-related fatigue. *Neurol Neuroimmunol Neuroinflamm*. (2018) 5:e423. doi: 10.1212/NXI.0000000000000423
118. Korzhova J, Bakulin I, Sinitsyn D, Poydasheva A, Suponeva N, Zakharova M, et al. High-frequency repetitive transcranial magnetic stimulation and intermittent theta-burst stimulation for spasticity management in secondary progressive multiple sclerosis. *Eur J Neurol*. (2019) 26:680–44. doi: 10.1111/ene.13877
119. Mori F, Codecà C, Kusayanagi H, Monteleone F, Boffa L, Rimano A, et al. Effects of intermittent theta burst stimulation on spasticity in patients with multiple sclerosis. *Eur J Neurol*. (2010) 17:295–300. doi: 10.1111/j.1468-1331.2009.02806.x
120. Liu M, Fan S, Xu Y, Cui L. Non-invasive brain stimulation for fatigue in multiple sclerosis patients: A systematic review and meta-analysis. *Mult Scler Relat Disord*. (2019) 36:101375. doi: 10.1016/j.msard.2019.08.017
121. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. (2011) 69:292–302. doi: 10.1002/ana.22366
122. Linnhoff S, Fiene M, Heinze H-J, Zaehle T. Cognitive fatigue in multiple sclerosis: an objective approach to diagnosis and treatment by transcranial electrical stimulation. *Brain Sci*. (2019) 9:100. doi: 10.3390/brainsci9050100

Conflict of Interest: FC has received travel grants from Biogen, Merck, and Sanofi-Genzyme.

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

frontiers in Neurology

BRIEF RESEARCH REPORT
published: 05 May 2020
doi: 10.3389/fneur.2020.00310

Gait and Functional Mobility in Multiple Sclerosis: Immediate Effects of Transcranial Direct Current Stimulation (tDCS) Paired With Aerobic Exercise

Giuseppina Pilloni[1,2]*, *Claire Choi*[3], *Giancarlo Coghe*[4], *Eleonora Cocco*[4], *Lauren B. Krupp*[1], *Massimiliano Pau*[2] and *Leigh E. Charvet*[1]

[1] NYU Langone Health, Department of Neurology, New York, NY, United States, [2] Department of Mechanical, Chemical and Materials Engineering, University of Cagliari, Cagliari, Italy, [3] SUNY Downstate, Department of Medicine, New York, NY, United States, [4] Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy

OPEN ACCESS

Edited by:
Moussa Antoine Chalah, Hôpitaux Universitaires Henri Mondor, France

Reviewed by:
Thorsten Rudroff, University of Iowa, United States
Fabian Steinberg, Louisiana State University, United States

***Correspondence:**
Giuseppina Pilloni
Giuseppina.Pilloni@nyulangone.org

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *25 February 2020*
Accepted: *31 March 2020*
Published: *05 May 2020*

Citation:
Pilloni G, Choi C, Coghe G, Cocco E, Krupp LB, Pau M and Charvet LE (2020) Gait and Functional Mobility in Multiple Sclerosis: Immediate Effects of Transcranial Direct Current Stimulation (tDCS) Paired With Aerobic Exercise. Front. Neurol. 11:310. doi: 10.3389/fneur.2020.00310

Walking impairments are a debilitating feature of multiple sclerosis (MS) because of the direct interference with daily activity. The management of motor symptoms in those with MS remains a therapeutic challenge. Transcranial direct current stimulation (tDCS) is a type of non-invasive brain stimulation that is emerging as a promising rehabilitative tool but requires further characterization to determine its optimal therapeutic use. In this randomized, sham-controlled proof-of-concept study, we tested the immediate effects of a single tDCS session on walking and functional mobility in those with MS. Seventeen participants with MS completed one 20-min session of aerobic exercise, randomly assigned to be paired with either active (2.5 mA, $n = 9$) or sham ($n = 8$) tDCS over the primary motor cortex (M1). The groups (active vs. sham) were matched according to gender (50% vs. 60% F), age (52.1 ± 12.85 vs. 54.2 ± 8.5 years), and level of neurological disability (median Expanded Disability Status Scale score 5.5 vs. 5). Gait speed on the 10-m walk test and the Timed Up and Go (TUG) time were measured by a wearable inertial sensor immediately before and following the 20-min session, with changes compared between conditions and time. There were no significant differences in gait speed or TUG time changes following the session in the full sample or between the active vs. sham groups. These findings suggest that a single session of anodal tDCS over M1 is not sufficient to affect walking and functional mobility in those with MS. Instead, behavioral motor response of tDCS is likely to be cumulative, and the effects of multiple tDCS sessions require further study.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier: NCT03658668.

Keywords: transcranial direct current stimulation, tDCS, non-invasive brain stimulation, multiple sclerosis, motor rehabilitation, gait, functional mobility, aerobic exercise

INTRODUCTION

Multiple sclerosis (MS) is the leading cause of progressive functional impairments in younger adults of working age (1). Multiple sclerosis symptoms are often variable across individuals and can affect motor, sensory, and cognitive functions (2). Loss of mobility is a key concern due to the interference with independence and the ability to complete activities of daily living (3, 4). Multiple factors contribute to the degeneration of MS ambulatory ability, such as muscle weakness, abnormal walking mechanisms, balance problems, spasticity, and fatigue (3, 5). While there is no typical pattern of MS gait disturbance, impairments often include reductions in gait velocity and step length (6, 7). Symptomatic treatment is an important topic for the management of MS (8, 9), with a strong unmet need for non-pharmacologic options to preserve and recover from MS-related walking impairments (10).

Non-invasive brain stimulation techniques, such as transcranial direct current stimulation (tDCS), are being studied for a range of applications in MS symptom management, including fatigue, cognitive deficits, neuropathic pain, and motor impairments (11). tDCS delivers weak electrical currents (1.0–2.5 mA) passed through electrodes (anode and cathode) placed on the scalp targeting brain regions of interest. This technique has been used to modulate the resting membrane potential in cortical and subcortical tissue promoting cell plasticity (12, 13). The neurophysiological response seems to be achieved through mechanisms of long-term potentiation or long-term depression of synapses (14, 15). tDCS can influence neural activity in a polarity-dependent manner: cortical excitability can be increased (under the anode) or reduced (under the cathode) in the underlying cortex (16).

Despite intense recent investigation of tDCS, parameters for dosing in terms of timing of application in relation to a paired training activity, current intensity, duration, and number of sessions remain largely undefined (17, 18). A growing number of studies, albeit with mixed results, have overall demonstrated the efficacy of a single anodal tDCS session over the primary motor cortex (M1) to improve motor performance in both healthy controls and patients with motor disorders (19–22). The probability to detect either neurophysiological or clinical responses still remains unclear, since treatment responses may be achieved by both single or repeated tDCS applications (23–26).

Previous findings have reported mixed effects after the application of tDCS over M1 on motor outcome variables (e.g., mobility and functionality of lower limbs, muscle strength, functional ambulation) in MS patients (27, 28). Given the current intensity delivered by tDCS is too low to generate *de novo* neuronal action potentials (29), the working mechanism is based on the "functional targeting" principle (30), where tDCS facilitates neuronal activation of specific pathways involved during the execution of a paired training activity. Therefore, potential interactions and synergies between tDCS and aerobic exercise have been recently studied to improve the recovery process within neurological conditions or to increase performance (31).

Aerobic exercise has demonstrated benefit in MS, with aerobic training shown to improve gait speed, stride length, and walking distance (32). Transcranial direct current stimulation may interact with exercise training enhancing the acute effect on motor functions and promote long-lasting benefits (31, 33). Thus, the use of tDCS during aerobic exercise may enhance the therapeutic effects via greater activation of neuroplastic mechanisms.

Specific electrode montages have varied across studies aimed at improving motor performance and symptoms to date, but most of them applied the anode over M1 area (34–36). Some studies conceptualized alternative motor electrode montages, varying electrode dimensions, and the position of the cathode, in order to optimize the stimulation of the lower limb motor cortex (37, 38). However, evidence is mixed as to whether these variations can improve effects compared to the standard motor montage (anode over M1 and cathode over the contralateral supraorbital area) [(37, 39)].

The current intensity has also varied across the studies, but 2.5 mA is the higher amperage of current clinical convention across trials (40, 41). Preliminary evidence and theoretical models (42, 43) provide support for the utilization of a relative higher stimulation amperage to increase cortical excitability. Moreover, previous studies have found good tolerability with higher amperage as the improved promotion of optimal and measurable response (44, 45).

The aim of this study was to test the motor response following a single session of tDCS over M1, clarifying its efficacy in enhancing the effect of aerobic exercise on walking and functional mobility performance in MS patients.

METHODS

Participants

Seventeen participants with either relapsing-remitting MS (RRMS) or secondary progressive MS (SPMS) were recruited. Eligibility criteria included ages 18–70 years, level of neurologic disability as measured by the Expanded Disability Status Scale (EDSS) (46) score from 1.0 to 6.5, and the ability to independently walk (with or without an assistive device) for at least 20 m. Potential participants were excluded if they had any history of brain trauma or seizures, any skin disorder or skin sensitive area near the stimulation locations, or were unable to understand the informed consent process and/or study procedures.

All participants provided written informed consent, and the study was conducted at the MS Comprehensive Care Center, NYU Langone Health. Ethical approval was obtained from the Institutional Review Board Committee of the New York University School of Medicine and followed the Ethical Principles for Medical Research Involving Human Subjects outlined in the Declaration of Helsinki.

Study Design and Experimental Protocol

This proof-of-concept study is part of a larger and ongoing clinical trial that employs a double-blind, sham-controlled, randomized design of tDCS paired with aerobic exercise. During the baseline visit, participants were screened for eligibility,

TABLE 1 | *Post-hoc* pairwise comparison of gait speed and TUG time pre-intervention and post-intervention for active and sham group.

	Active group ($n = 9$)			Sham group ($n = 8$)		
	Pre-intervention	**Post-intervention**	**Comparison pre vs. post *p*-value**	**Pre-intervention**	**Post-intervention**	**Comparison pre vs. post *p*-value**
Gait speed (m/s)	0.92 ± 0.31	0.95 ± 0.32	0.456	0.96 ± 0.35	0.96 ± 0.34	0.558
TUG time (s)	14.48 ± 4.11	14.34 ± 4.02	0.195	15.19 ± 4.56	14.58 ± 4.33	0.103

Values are reported as mean ± SD.

consented, and randomized to the active or sham arm within strata defined by EDSS level [EDSS "low" (0–3.5) vs. "high" (4.0–6.5) score] and age (18–45 years vs. 46–65 years). To ensure the double-blind nature of the study (both patient and the technician involved in treatment and assessment), an independent technician completed the randomization and pre-programmed the tDCS device in advance to deliver active or sham stimulation accordingly.

Intervention: tDCS Paired With Aerobic Exercise

For the current analyses, we analyzed gait and functional mobility measures before and after the first tDCS + exercise session. Both the active and sham participants completed 20 min of stimulation during exercise using a recumbent combination arm/leg elliptical ergometer (PhysioStep LTX-700). The exercise period included heart rate (HR) monitoring via Fitbit wristband (Fitbit Inc., California, USA) to ensure that each participant met the recommended target HR for the physical exercise for MS, training at moderate intensity corresponding to 60–80% of age-predicted maximum HR (47).

Transcranial direct current stimulation was applied to the M1 cortex with the goal of enhancing the activation of the cortical pathways involved and activated during pedaling/cycling (48, 49). Active and sham tDCS was delivered using the 1 × 1 tDCS mini-clinical trial device (mini-CT; Soterix Medical Inc., New York, NY, USA) using an optimized motor montage targeting the M1 area with supraorbital exit (C3 anode/Fp2 cathode according to 10/20 EEG), with two pre-saturated sponge surface electrodes (square shape, 5 × 5 cm^2). The current intensity was set at 2.5 mA, with the goal of increasing cortical excitability to promote optimal and measurable clinical response (42, 43, 45).

All study procedures were the same for active and sham conditions. For the active participants, the current intensity was set at 2.5 mA for the entire session. For the sham participants, and following the current blinding recommendations, the device delivered a 60-s ramp up/down to the 2.5-mA target at the beginning and end of the 20-min period, with no other current delivery. In this way, the sham procedure produces similar sensory experiences to mask the stimulation condition administered (50, 51).

Motor Assessment, Blinding Assessment, and Motor Outcomes

To measure changes in gait and functional mobility, the instrumented 10-m walking test and the instrumented Timed Up and Go (TUG) test were measured using a single wearable inertial sensor (G-Sensor®; BTS Bioengineering S.p.A., Milan, Italy). Both tests were performed twice consecutively, the first time for familiarization, and the second time for data capture. The inertial sensor was positioned to the participant's waist using a semielastic belt (covering the L4–L5 intervertebral disc for walking assessment and L1-L2 for TUG test providing acceleration values along three orthogonal axes and transmitted via Bluetooth to a PC, where the raw accelerations were processed. For the 10-m walking test, participants were instructed to walk along a 10-m path at their typical speed. For the TUG, participants were instructed to sit on a standard armless chair with back support. At start, they stood up, walked for 3 m at self-selected speed, performed a 180° turn around at a cone, and then walked back to the chair, and then performed a second 180° turn to sit down.

Post-processing of the acceleration signals using dedicated software (BTS G-Studio; BTS Bioengineering S.p.A.) allowed computing the following parameters:

- 10-m walking test:
  - the mean velocity of progression (m/s);
- TUG test:
  - TUG time: the time needed to complete the test (s).

Both the 10-m walk test and the TUG are validated as standard clinical tests with high test–retest reliability for measuring walking function and functional mobility in patients with MS (52, 53).

At the end of the study, blinding integrity was assessed by asking the participants to guess the received treatment.

Statistical Analysis

The collected data were analyzed using the statistical package SPSS version 25 (SPSS, Inc., Chicago, IL, USA). Descriptive analyses were generated for demographic and clinical variables of the two arms. The normal distribution of the dependent variables (gait velocity and TUG time) was assessed by the Kolmogorov–Smirnov test. The dependent variables of the study met the criteria of normality. Because of the normal distribution, a general mixed-model analysis of variance (ANOVA) 2 × 2 (intervention × time) was performed to examine the effect of the between-subjects factor treatment (active, sham) and the within-subjects factor time (pre-assessment and post-assessment). The type I error (α) was set at 0.05, and the effect sizes were assessed using the η^2 coefficient. When a significant main effect was reached, *post-hoc* tests with Sidak correction for

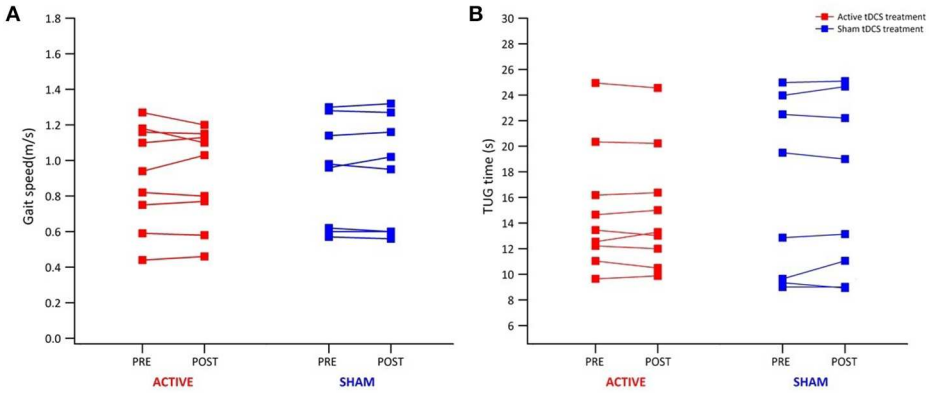


FIGURE 1 | Individual results from 10-m walk test and TUG test for all participants. Individual results of each participant of the active (red) and sham group (blue) before and immediately after tDCS paired with aerobic exercise for gait speed **(A)** and TUG time **(B)**.

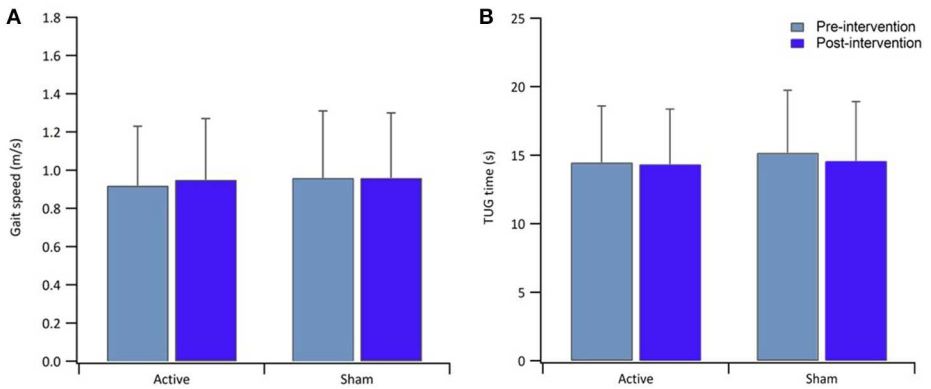


FIGURE 2 | Bar graphs show result of gait speed **(A)** and TUG time **(B)** for the active and sham group, pre-intervention (light blue) and post-intervention (blue). Error bars in both graphs indicate ± SD.

multiple comparisons were conducted to assess treatment or time point differences.

RESULTS

The groups were matched in terms of demographic and clinical features (50 vs. 60% *F*; median EDSS: 5.5 vs. 5; 2 RRMS, 7 SPMS vs. 3 RRMS, 5 SPMS; age 52.1 ± 12.85 vs. 54.2 ± 8.5 years, active vs. sham respectively, all $p > 0.05$).

Stimulation was well-tolerated across participants and with side effects of itching, tingling, and head pain. No side effect reached an intensity level of >7 (rated on a 0- to 10-point scale) for any participant, and all side effects resolved at the end of the stimulation period.

Overall, the tDCS condition assignment (active, sham) was identified correctly by 28% of participants (specifically, the 33% of the participants in the sham group). The result of the blinding integrity is in agreement with the standards suggested in previous studies (51, 54).

All participants completed the 20-min aerobic exercise maintaining the targeted moderate level. The average HR during the session was 110.9 ± 4.0 beats/min.

Table 1 reports data of gait velocity and TUG time as mean ± SD. The ANOVA test indicated no significant changes in gait speed and TUG time after one single session in either the whole group or active vs. sham tDCS (**Figures 1**, **2**). There were no significant main effects of the intervention [$F_{(1,15)} = 0.074$, $p = 0.861$, $\eta^2 = 0.006$; $F_{(1,15)} = 0.087$, $p = 0.883$, $\eta^2 = 0.002$], as well as of the time [$F_{(1,15)} = 2.346$, $p = 0.070$, $\eta^2 = 0.101$; $F_{(1,15)} = 1.784$, $p = 0.239$, $\eta^2 = 0.088$] and time × intervention interactions [$F_{(1,15)} = 1.946$, $p = 0.115$, $\eta^2 = 0.093$; $F_{(1,15)} = 1.381$, $p = 0.446$, $\eta^2 = 0.038$] for gait speed and TUG time, respectively. These findings indicate no immediate effect on walking and functional mobility performance with either tDCS paired with aerobic exercise or aerobic exercise alone.

DISCUSSION

The aim of this study was to investigate the immediate effects of a single tDCS session over M1 on the behavioral motor responses of patients with MS. We did not find any significant treatment effect in walking speed and TUG time following the application of a single session of tDCS paired with aerobic exercise. While changes have been reported following a single

session in prior studies (19, 25), our findings are consistent with the growing literature across neurological disorders indicating that a single session of tDCS is not enough to lead to meaningful or measurable behavioral outcomes (24, 55) or to enhance the benefits of physical training on motor functions in those with MS.

Previously, the effects of a single tDCS application over M1 on gait in MS (56) during the 6-min walk test (2 mA, 6 min) did not report any improvement in distance walked, gait velocity, and stride length. However, this study was limited by the use of a short duration of the stimulation (6 min) (56), while the present study adopted a longer duration of stimulation equal to 20 min according to recommendations based on evidence to date (40, 41).

Our findings are consistent with another previous report of tDCS and hand functioning (23) in MS, where they explored the effect of a single session of anodal tDCS applied to the M1 contralateral to the affected hand. The authors reported an increase in the corticospinal output and projection strengthening evaluated by using transcranial magnetic stimulation, but no behavioral motor effects were measured (23).

The current results do not necessarily imply the absence of an increase activation of the underlying brain region. An enhancement of MEP amplitudes, corticospinal output, and projection strength has been reported with a single application, even in the absence of measurable behavioral or clinical outcomes (23, 57).

When targeting behavioral outcomes, dosing dimensions such as the specific current intensity, duration of the stimulation, and number of sessions, or response variability remain unknown. It is important to consider pairing tDCS with behavioral training or rehabilitative activity as a critical dimension of dosing (31, 35, 58, 59) to improve motor outcomes. This multimodal approach is likely to have stronger effects on promoting synaptic changes and increasing the likelihood of detecting behavioral motor responses.

Specific to stimulation intensity and duration, findings are mixed (45, 60). We chose the conventional 20 min of stimulation at the higher 2.5mA current intensity under the hypothesis that these parameters would lead to higher brain activation (42); however, tDCS dosing may not necessarily be linear. In fact, either increasing the current intensity >4 mA or prolonging the stimulation more than 30-min duration is not always accompanied by an increase of its efficacy, with either change in the direction potentially leading to different patterns of neuronal activation (61, 62). It may be that alternative M1 (or other) montages as well as stimulation intensity could have resulted in different findings.

Nonetheless, the absence of effect of one tDCS application in MS for motor outcomes is an important finding as many studies continue to evaluate the clinical responses of a single session of tDCS. Recent work in MS (28) indicates that, in MS, multiple stimulation sessions can lead to benefit. In a sample of $n = 13$, those who received anodal tDCS stimulation over the M1 walked faster during the Timed 25-Foot Walk after seven sessions (28). The number of overall applications may be key in evaluating its rehabilitative and restorative potential according to the consolidation effects (63–65).

Limitations of the current study include its relatively small sample size. With a larger sample or greater range of MS participants, it is possible that more subtle effects of an initial tDCS application could be detected. In addition, while one strength of our study is the use of an advance technology to detect and characterize motor outcomes, we were not able to correspond findings to actual neurophysiological measures (e.g., structural and metabolic analysis of brain functions in response to the stimulation). The study would also have been strengthened by including a condition with tDCS only (without exercise) as an additional comparison.

Future studies need to more clearly define the effectiveness of tDCS as treatment option or as therapeutic adjuvant in motor rehabilitation. Clinical studies need to be designed to clarify the dimensions of dosing, not only including number of sessions, current intensity, and electrode montage, but also exploring other dosing dimensions represented by the combination of the practice of motor task or physical training and its timing of application (before/during/after stimulation). It would also be important to integrate the acquisition of functional neuroimaging (e.g., functional magnetic resonance imaging, positron emission tomography) with tDCS, to better understand how the stimulation modulates ongoing brain activity and connectivity.

CONCLUSION

Taken together, these findings indicate that a single session of anodal tDCS over M1 is not sufficient to improve walking and functional mobility in MS. Instead, behavioral effects of tDCS are likely to be cumulative, with a single session of tDCS able to provoke neurophysiological changes. Future studies with multiple and repeated sessions and paired with motor training are warranted in order to test the cumulative response in neural excitability outlasting the stimulation period to determine optimal clinical utilization.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available to any qualified researcher on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board Committee of the New York University School of Medicine. Study procedures were conducted in accordance with the Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GP, LC, MP, EC, and GC designed the study. Participants were recruited by CC and GP, and screened by LK. GP performed data

collection and analysis. GP and LC interpreted the results. The manuscript was drafted by GP and LC. All the authors critically revised the manuscript and approved the final version.

FUNDING

This study was supported by the Lourie Foundation, Inc.

REFERENCES

1. Compston A, Coles A. Multiple sclerosis. *Lancet*. (2002) 359:1221–31. doi: 10.1016/S0140-6736(02)08220-X
2. Ghasemi N, Razavi S, Nikzad E. Multiple sclerosis: pathogenesis, symptoms, diagnoses and cell-based therapy. *Cell J*. (2017) 19:1–10. doi: 10.22074/cellj.2016.4867
3. Cameron MH, Wagner JM. Gait abnormalities in multiple sclerosis: pathogenesis, evaluation, and advances in treatment. *Curr Neurol Neurosci Rep*. (2011) 11:507–15. doi: 10.1007/s11910-011-0214-y
4. Larocca NG. Impact of walking impairment in multiple sclerosis: perspectives of patients and care partners. *Patient*. (2011) 4:189–201. doi: 10.2165/11591150-000000000-00000
5. Sosnoff JJ, Socie MJ, Boes MK, Sandroff BM, Pula JH, Suh Y, et al. Mobility, balance and falls in persons with multiple sclerosis. *PLoS ONE*. (2011) 6:e28021. doi: 10.1371/journal.pone.0028021
6. Cattaneo D, Regola A, Meotti M. Validity of six balance disorders scales in persons with multiple sclerosis. *Disabil Rehabilit*. (2006) 28:789–95. doi: 10.1080/09638280500404289
7. Kelleher KJ, Spence W, Solomonidis S, Apatsidis D. The characterisation of gait patterns of people with multiple sclerosis. *Disabil Rehabil*. (2010) 32:1242–50. doi: 10.3109/09638280903464497
8. Kesselring J, Beer S. Symptomatic therapy and neurorehabilitation in multiple sclerosis. *Lancet Neurol*. (2005) 4:643–52. doi: 10.1016/S1474-4422(05)70193-9
9. Comi G, Radaelli M, Soelberg Sorensen P. Evolving concepts in the treatment of relapsing multiple sclerosis. *Lancet*. (2017) 389:1347–56. doi: 10.1016/S0140-6736(16)32388-1
10. Dunn J, Blight A. Dalfampridine: a brief review of its mechanism of action and efficacy as a treatment to improve walking in patients with multiple sclerosis. *Curr Med Res Opin*. (2011) 27:1415–23. doi: 10.4137/JCNSD.S4868
11. Ayache SS, Chalah MA. The place of transcranial direct current stimulation in the management of multiple sclerosis-related symptoms. *Neurodegener Dis Manag*. (2018) 8:411–22. doi: 10.2217/nmt-2018-0028
12. Woods A, Antal A, Bikson M, Boggio P, Brunoni A, Celnik P, et al. A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clin Neurophysiol*. (2016) 127:1031–48. doi: 10.1016/j.clinph.2015.11.012
13. Lefaucheur JP, Antal A, Ayache SS, Benninger DH, Brunelin J, Cogiamanian F, et al. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). *Clin Neurophysiol*. (2017) 128:56–92. doi: 10.1016/j.clinph.2016.10.087
14. Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG,et al. Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron*. (2010) 66:198–204. doi: 10.1016/j.neuron.2010.03.035
15. Kronberg G, Bridi M, Abel T, Bikson M, Parra LC. Direct current stimulation modulates LTP and LTD: activity dependence and dendritic effects. *Brain Stimul*. (2017) 10:51–8. doi: 10.1016/j.brs.2016.10.001
16. Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol Lond*. (2000) 527:633–9. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x
17. Goldsworthy MR, Hordacre B. Dose dependency of transcranial direct current stimulation: implications for neuroplasticity induction in health and disease. *J Physiol Lond*. (2017) 595:3265–6. doi: 10.1113/JP274089
18. Callan D, Perrey S. The use of tDCS and rTMS methods in neuroergonomics. In: *The Neuroergonomics: The Brain at Work and in Everyday Life*, eds H. Ayaz and F. Dehais. Amsterdam: Elsevier (2019) p. 31–3. doi: 10.1016/B978-0-12-811926-6.00005-1
19. Grecco LA, Duarte NA, Zanon N, Galli M, Fregni F, Oliveira CS. Effect of a single session of transcranial direct-current stimulation on balance and spatiotemporal gait variables in children with cerebral palsy: a randomized sham-controlled study. *Braz J Phys Ther*. (2014) 18;419–27. doi: 10.1590/bjpt-rbf.2014.0053
20. Saruco E, Di Rienzo F, Nunez-Nagy S, Rubio-Gonzalez MA, Jackson PL, Collet C, et al. Anodal tDCS over the primary motor cortex improves motor imagery benefits on postural control: a pilot study. *Sci Rep*. (2017) 7:480. doi: 10.1038/s41598-017-00509-w
21. Inguaggiato E, Bolognini N, Fiori S, Cioni G. Transcranial direct current stimulation (tDCS) in unilateral cerebral palsy: a pilot study of motor effect. *Neural Plast*. (2019) 2019:2184398. doi: 10.1155/2019/2184398
22. Sanchez-Kuhn A, Perez-Fernandez C, Canovas R, Flores P, Sanchez-Santed F. Transcranial direct current stimulation as a motor neurorehabilitation tool: an empirical review. *Biomed Eng Online*. (2017) 16(Suppl. 1):76. doi: 10.1186/s12938-017-0361-8
23. Cuypers K, Leenus DJ, Van Wijmeersch B, Thijs H, Levin O, Swinnen SP, et al. Anodal tDCS increases corticospinal output and projection strength in multiple sclerosis. *Neurosci Lett*. (2013) 554:151–5. doi: 10.1016/j.neulet.2013.09.004
24. Meesen RL, Thijs H, Leenus DJ, Cuypers KJR. A single session of 1 mA anodal tDCS-supported motor training does not improve motor performance in patients with multiple sclerosis. *Restor Neurol Neurosci*. (2014) 32:293–300. doi: 10.3233/RNN-130348
25. Tahtis V, Kaski D, Seemungal BM. The effect of single session bi-cephalic transcranial direct current stimulation on gait performance in sub-acute stroke: a pilot study. *Restor Neurol Neurosci*. (2014) 32:527–32. doi: 10.3233/RNN-140393
26. Hashemirad F, Zoghi M, Fitzgerald PB, Jaberzadeh S. The effect of anodal transcranial direct current stimulation on motor sequence learning in healthy individuals: a systematic review and meta-analysis. *Brain Cogn*. (2016) 102:1–12. doi: 10.1016/j.bandc.2015.11.005
27. Iodice R, Dubbioso R, Ruggiero L, Santoro L, Manganelli F. Anodal transcranial direct current stimulation of motor cortex does not ameliorate spasticity in multiple sclerosis. *Restor Neurol Neurosci*. (2015) 33:487–92. doi: 10.3233/RNN-150495
28. Oveisgharan S, Karimi Z, Abdi S, Sikaroodi H. The use of brain stimulation in the rehabilitation of walking disability in patients with multiple sclerosis: a randomized double-blind clinical trial study. *Iranian J Neurol*. (2019) 18:57–63.
29. Jackson MP, Rahman A, Lafon B, Kronberg G, Ling D, Parra LC, et al. Animal models of transcranial direct current stimulation: Methods and mechanisms. *Clin Neurophysiol*. (2016) 127:3425–54. doi: 10.1016/j.clinph.2016.08.016
30. Bikson M, Rahman A. Origins of specificity during tDCS: anatomical, activity-selective, and input-bias mechanisms. *Front Hum Neurosci*. (2013) 7:688. doi: 10.3389/fnhum.2013.00688
31. Steinberg F, Pixa NH, Fregni F. A review of acute aerobic exercise and transcranial direct current stimulation effects on cognitive functions and their potential synergies. *Front Hum Neurosci*. (2019) 12:534. doi: 10.3389/fnhum.2018.00534
32. Snook EM, Motl RW. Effect of exercise training on walking mobility in multiple sclerosis: a meta-analysis. *Neurorehabil Neural Repair*. (2009) 23:108–16. doi: 10.1177/1545968308320641
33. Moreau D, Wang CH, Tseng P, Juan CH. Blending transcranial direct current stimulations and physical exercise to maximize cognitive improvement. *Front Psychol*. (2015) 6:678. doi: 10.3389/fpsyg.2015.00678
34. Nitsche MA, Schauenburg A, Lang N, Liebetanz D, Exner C, Paulus W, et al. Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. *J Cogn Neurosci*. (2003) 15:619–26. doi: 10.1162/089892903321662994
35. Morya E, Monte-Silva K, Bikson M, Esmaeilpour Z, Biazoli CE Jr, Fonseca A, et al. Beyond the target area: an integrative view of tDCS-induced motor cortex modulation in patients and athletes. *J Neuroeng Rehabil*. (2019) 16:141. doi: 10.1186/s12984-019-0581-1

36. Santos Ferreira I, Teixeira Costa B, Lima Ramos C, Lucena P, Thibaut A, Fregni F. Searching for the optimal tDCS target for motor rehabilitation. *J Neuroeng Rehabil*. (2019) 16:90. doi: 10.1186/s12984-019-0561-5
37. Foerster ÁS, Rezaee Z, Paulus W, Nitsche MA, Dutta A. Effects of cathode location and the size of anode on anodal transcranial direct current stimulation over the leg motor area in healthy humans. *Front Neurosci*. (2018) 12:443. doi: 10.3389/fnins.2018.00443
38. Foerster Á, Dutta A, Kuo MF, Paulus W, Nitsche MA. Effects of anodal transcranial direct current stimulation over lower limb primary motor cortex on motor learning in healthy individuals. *Eur J Neurosci*. (2018) 47:779–89. doi: 10.1111/ejn.13866
39. Patel R, Madhavan S. Comparison of transcranial direct current stimulation electrode montages for the lower limb motor cortex. *Brain Sci*. (2019) 9:189. doi: 10.3390/brainsci9080189
40. Fregni F, Nitsche MA, Loo CK, Brunoni AR, Marangolo P, Leite J, et al. Regulatory considerations for the clinical and research use of transcranial direct current stimulation (tDCS): review and recommendations from an expert panel. *Clin Res Regul Aff*. (2015) 32:22–35. doi: 10.3109/10601333.2015.980944
41. Charvet LE, Shaw MT, Bikson M, Woods AJ, Knotkova H. Supervised transcranial direct current stimulation (tDCS) at home: a guide for clinical research and practice. *Brain Stimul*. (2020) 13:686–93. doi: 10.1016/j.brs.2020.02.011
42. Bikson M, Inoue M, Akiyama H, Deans JK, Fox JE, Miyakawa H, et al. Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices *in vitro*. *J Physiol*. (2004) 557:175–90. doi: 10.1113/jphysiol.2003.055772
43. Khadka N, Borges H, Paneri B, Kaufman T, Nassis E, Zannou AL, et al. Adaptive current tDCS up to 4mA. *Brain Stimul*. (2020) 13:69–79. doi: 10.1016/j.brs.2019.07.027
44. Pilloni G, Shaw M, Feinberg C, Clayton A, Palmeri M, Datta A, et al. Long term at-home treatment with transcranial direct current stimulation (tDCS) improves symptoms of cerebellar ataxia: a case report. *J Neuroengineer Rehabil*. (2019) 16:41. doi: 10.1186/s12984-019-0514-z
45. Workman CD, Kamholz J, Rudroff T. The tolerability and efficacy of 4 mA transcranial direct current stimulation on leg muscle fatigability. *Brain Sci*. (2020) 10:12. doi: 10.3390/brainsci10010012
46. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. (1983) 33:1444–52. doi: 10.1212/wnl.33.11.1444
47. Dalgas U, Stenager E, Ingemann-Hansen T. Multiple sclerosis and physical exercise: recommendations for the application of resistance-, endurance- and combined training. *Multiple Scler J*. (2008) 14:35–53. doi: 10.1177/1352458507079445
48. Jain S, Gourab K, Schindler-Ivens S, Schmit BD. EEG during pedaling: evidence for cortical control of locomotor tasks. *Clin Neurophysiol*. (2013) 124:379–90. doi: 10.1016/j.clinph.2012.08.021
49. Tatemoto T, Tanaka S, Maeda K, Tanabe S, Kondo K, Yamaguchi T. Skillful cycling training induces cortical plasticity in the lower extremity motor cortex area in healthy persons. *Front Neurosci*. (2019) 13:927. doi: 10.3389/fnins.2019.00927
50. Palm U, Reisinger E, Keeser D, Kuo MF, Pogarell O, Leicht G, et al. Evaluation of sham transcranial direct current stimulation for randomized, placebo-controlled clinical trials. *Brain Stimul*. (2013) 6:690–5. doi: 10.1016/j.brs.2013.01.005
51. Dinn W, Göral F, Adigüzel S, Karamürsel S, Fregni F, Aycicegi-Dinn A, et al. Effectiveness of tDCS blinding protocol in a sham-controlled study. *Brain Stimul*. (2017) 10:401. doi: 10.3390/brainsci8020037
52. Bethoux F, Bennett S. Evaluating walking in patients with multiple sclerosis: which assessment tools are useful in clinical practice? *Int J MS Care*. (2011) 13:4–14. doi: 10.7224/1537-2073-13.1.4
53. Sebastião E, Sandroff BM, Learmonth YC, Motl RW. Validity of the timed up and go test as a measure of functional mobility in persons with multiple sclerosis. *Archiv Phys Med Rehabil*. (2016) 97:1072–7. doi: 10.1016/j.apmr.2015.12.031
54. Brunoni AR, Schestatsky P, Lotufo PA, Benseñor IM, Fregni F. Comparison of blinding effectiveness between sham tDCS and placebo sertraline in a 6-week major depression randomized clinical trial. *Clin Neurophysiol*. (2014) 125:298–305. doi: 10.1016/j.clinph.2013.07.020
55. Proessl F, Poston B, Rudroff T. Does a single application of anodal tDCS improve knee extensor fatigability in people with multiple sclerosis? *Brain Stimul*. (2018) 11:1388–90. doi: 10.1016/j.brs.2018.08.005
56. Workman CD, Kamholz J, Rudroff T. Transcranial Direct Current Stimulation (tDCS) to improve gait in multiple sclerosis: a timing window comparison. *Front Hum Neurosci*. (2019) 13:420. doi: 10.3389/fnhum.2019.00420
57. Agboada D, Samani MM, Jamil A, Kuo MF, Nitsche MA. Expanding the parameter space of anodal transcranial direct current stimulation of the primary motor cortex. *Sci Rep*. (2019) 9:18185. doi: 10.1038/s41598-019-54621-0
58. Charvet LE, Dobbs B, Shaw MT, Bikson M, Datta A, Krupp LB. Remotely supervised transcranial direct current stimulation for the treatment of fatigue in multiple sclerosis: results from a randomized, sham-controlled trial. *Mult Scler*. (2017) 24:1760–9. doi: 10.1177/1352458517732842
59. Charvet L, Shaw M, Dobbs B, Frontario A, Sherman K, Bikson M, et al. Remotely supervised transcranial direct current stimulation increases the benefit of at-home cognitive training in multiple sclerosis. *Neuromodulation*. (2017) 21:383–9. doi: 10.1111/ner.12583
60. Tremblay S, Larochelle-Brunet F, Lafleur LP, El Mouderrib S, Lepage JF, Théoret H. Systematic assessment of duration and intensity of anodal transcranial direct current stimulation on primary motor cortex excitability. *Eur J Neurosci*. (2016) 44:2184–90. doi: 10.1111/ejn.13321
61. Batsikadze G, Moliadze V, Paulus W, Kuo MF, Nitsche MA. Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. *Jour Phys*. (2013) 591:1987–2000. doi: 10.1113/jphysiol.2012.249730
62. Nitsche MA, Bikson M. Extending the parameter range for tDCS: safety and tolerability of 4 mA stimulation. *Brain Stimul*. (2017) 10:541. doi: 10.1016/j.brs.2017.03.002
63. Reis J, Schambra HM, Cohen LG, Buch E, Fritsch B, Zarahn E, et al. (2009). Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proc Natl Acad Sci U.S.A*. 106, 1590–1595. doi: 10.1073/pnas.0805413106
64. Monte-Silva K, Kuo MF, Hessenthaler S, Fresnoza S, Liebetanz D, Paulus W, et al. Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. *Brain Stimul*. (2013) 6:424–32. doi: 10.1016/j.brs.2012.04.011
65. Shaw M, Pilloni G, Charvet L. Delivering transcranial direct current stimulation away from clinic: remotely supervised tDCS. *Mil Med*. (2020) 185(Suppl. 1):319–25. doi: 10.1093/milmed/usz348

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

frontiers in Neurology

ORIGINAL RESEARCH
published: 05 May 2020
doi: 10.3389/fneur.2020.00193

Probing Context-Dependent Modulations of Ipsilateral Premotor-Motor Connectivity in Relapsing-Remitting Multiple Sclerosis

Elisa Ruiu[1,2†], *Raffaele Dubbioso*[1,3†], *Kristoffer Hougaard Madsen*[1,4], *Olivia Svolgaard*[1], *Estelle Raffin*[1,5], *Kasper Winther Andersen*[1], *Anke Ninija Karabanov*[1,6*‡] *and Hartwig Roman Siebner*[1,7,8‡]

[1] Danish Research Centre for Magnetic Resonance, Centre for Functional and Diagnostic Imaging and Research, Copenhagen University Hospital Hvidovre, Section 714, Hvidovre, Denmark, [2] Department of Neurology, University Hospital of Sassari, Sassari, Italy, [3] Department of Neurosciences, Reproductive Sciences and Odontostomatology, University Federico II of Naples, Naples, Italy, [4] Section for Cognitive Systems, Department of Applied Mathematics and Computer Science, Technical University of Denmark, Lyngby, Denmark, [5] Brain Mind Institute and Centre of Neuroprosthetics, Swiss Federal Institute of Technology (EPFL), Geneva, Switzerland, [6] Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, Denmark, [7] Department of Neurology, Copenhagen University Hospital Bispebjerg, Copenhagen, Denmark, [8] Faculty of Medical and Health Sciences, Institute for Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

Objective: We employed dual-site TMS to test whether ipsilateral functional premotor-motor connectivity is altered in relapsing-remitting Multiple Sclerosis (RR-MS) and is related to central fatigue.

Methods: Twelve patients with RR-MS and 12 healthy controls performed a visually cued Pinch-NoPinch task with their right hand. During the reaction time (RT) period of Pinch and No-Pinch trials, single-site TMS was applied to the left primary motor cortex (M1) or dual-site TMS was applied to the ipsilateral dorsal premotor cortex (PMd) and to M1. We traced context-dependent changes of corticospinal excitability and premotor–motor connectivity by measuring Motor-Evoked Potentials (MEPs) in the right first dorsal interosseus muscle. Central fatigue was evaluated with the Fatigue Scale for Motor and Cognitive Functions (FSMS).

Results: In both groups, single-pulse TMS revealed a consistent increase in mean MEP amplitude during the Reaction Time (RT) period relative to a resting condition. Task-related corticospinal facilitation increased toward the end of the RT period in Pinch trials, while it decreased in No-Pinch trials. Again, this modulation of MEP facilitation by trial type was comparable in patients and controls. Dual-site TMS showed no significant effect of a conditioning PMd pulse on ipsilateral corticospinal excitability during the RT period in either group. However, patients showed a trend toward a relative attenuation in functional PMd-M1 connectivity at the end of the RT period in No-Pinch trials, which correlated positively with the severity of motor fatigue ($r = 0.69$; $p = 0.007$).

OPEN ACCESS

Edited by:
Samar S. Ayache,
Hôpitaux Universitaires Henri Mondor, France

Reviewed by:
Pablo Billeke,
Universidad del Desarrollo, Chile
Grigorios Nasios,
University of Ioannina, Greece

***Correspondence:**
Anke Ninija Karabanov
ankenk@drcmr.dk

†These authors share first authorship

‡These authors share last authorship

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: 11 December 2019
Accepted: 02 March 2020
Published: 05 May 2020

Citation:
Ruiu E, Dubbioso R, Madsen KH, Svolgaard O, Raffin E, Andersen KW, Karabanov AN and Siebner HR (2020) Probing Context-Dependent Modulations of Ipsilateral Premotor-Motor Connectivity in Relapsing-Remitting Multiple Sclerosis. Front. Neurol. 11:193. doi: 10.3389/fneur.2020.00193

Conclusions: Dynamic regulation of corticospinal excitability and ipsilateral PMd-M1 connectivity is preserved in patients with RR-MS. MS-related fatigue scales positively with an attenuation of premotor-to-motor functional connectivity during cued motor inhibition.

Significance: The temporal, context-dependent modulation of ipsilateral premotor-motor connectivity, as revealed by dual-site TMS of ipsilateral PMd and M1, constitutes a promising neurophysiological marker of fatigue in MS.

Keywords: multiple sclerosis, dual-site TMS, fatigue, movement preparation, dorsal premotor cortex, primary motor cortex

HIGHLIGHTS

- Dynamic modulation of ipsilateral premotor-to-motor cortical drive was probed with TMS.
- Patients with relapsing-remitting multiple sclerosis showed normal modulation during a cued Pinch-NoPinch task.
- Attenuation of premotor-to-motor drive in a NoPinch context scaled positively with motor fatigue in patients.
- Context-dependent attenuation of premotor-to-motor drive may contribute to motor fatigue in multiple sclerosis.

INTRODUCTION

Multiple Sclerosis (MS) is the most common autoimmune disorder of the central nervous system (CNS) (1), and its pathology includes both axonal damage and demyelination (2). A majority of MS patients initially exhibit a relapsing-remitting disease course (RR-MS), characterized by attacks with acutely emerging focal neurological deficits that totally or partially recover over the following weeks. Relapses can cause a large variety of classic neurological deficits, affecting motor and sensory function, but also "less quantifiable" symptoms such as excessive motor or cognitive fatigue (3).

Transcranial magnetic stimulation (TMS) allows corticomotor excitability to be quantified by recording motor evoked potentials (MEP) and is widely used to characterize cortico-motor dysfunction in MS (4, 5). Single-pulse TMS studies have demonstrated abnormal corticospinal excitability and connectivity in patients with RR-MS even during relapse-free periods (6). Single-pulse studies also found that basic MEP-based excitability measures scale with individual motor function or disability scores (7, 8). Paired-pulse TMS, which applies a conditioning and test pulse with the same coil, has been used to probe intracortical excitability in RR-MS and has revealed a link between individual disability and measures of intracortical inhibition and intracortical facilitation (8, 9). These correlations between intracortical excitability and disability score at the single-patient level may be obscured when pooling patients (10), highlighting the importance of taking inter-individual variations into account when investigating a heterogeneous disease like multiple sclerosis (11).

TMS can also be used to investigate the neurobiological mechanisms underlying specific motor symptoms like motor fatigue, which represents one of the most common symptoms in MS (12). One study linked motor fatigue to alterations of intracortical excitability in patients with RR-MS while the patients were at rest (13). Other TMS studies used single-pulse TMS to probe corticospinal excitability during the performance of a simple, visually cued reaction time task. These studies revealed a reduction of pre-movement facilitation that correlated with individual fatigue scores (14, 15), pointing to an impaired initialization of movements. Complementing these task-related TMS studies, task-related functional brain imaging studies found excessive recruitment of higher-order premotor areas, such as the dorsal premotor cortex (16–18). The premotor activity may reflect excessive volitional drive in the context of inefficient movement initiation.

Dual-site TMS (dsTMS) has been successfully used to probe the effective connectivity of pathways projecting from cortical or cerebellar brain regions to the precentral primary motor cortex (19–22). These dsTMS paradigms apply a conditioning stimulus (CS) over the remote motor area and give a test stimulus (TS) over the primary motor hand area (M1-HAND) to probe the effect of the conditioning stimulus on corticospinal excitability. Ipsilateral premotor-to-primary motor connectivity can be probed with optimized small TMS coils, which apply the CS over the dorsal premotor cortex (PMd) and the test stimulus (TS) over ipsilateral M1-HAND (23, 24). Groppa et al. introduced a dsTMS paradigm in which the TS is applied over M1-HAND 0.8–2.0 ms before a CS over ipsilateral PMd (23, 24). The premotor CS facilitates corticospinal excitability in ipsilateral M1-HAND via an ultra-fast premotor-to-motor pathway. Functional premotor-to-motor interaction, as probed by this dsTMS paradigm, dynamically changed depending on the motor context (23, 24). When healthy individuals performed a two-choice Go-NoGo

Abbreviations: CNS, Central Nervous System; CS, Conditioning Stimulus; DMSC, Danish Multiple Sclerosis Centre; dsTMS, dual-site TMS; EDSS, Expanded Disability Status Scale; EMG, Electromyography; FDI, First Dorsal Interosseous; FSMC, Fatigue Scale for Motor and Cognitive Functions; HC, Healthy Controls; ISI, Interstimulus Interval; M1, Primary Motor Cortex; $M1_{HAND}$, Primary Motor Hand Area; MCV, Maximum Voluntary Contraction; MEP, Motor Evoked Potential; MS, Multiple Sclerosis; PASAT, Paced Auditory Serial Addition Test; PEST, Parameter Estimation in Sequential Test; PMd, dorsal Premotor Cortex; PMd-M1, Premotor-Motor; RMT, Resting Motor Threshold; RR, Relapsing-Remitting; RT, Response Time; SD, Standard Error; SDMT, Symbol Digit Modalities Test; SEM, Standard Error of the Mean; SICF, Short Intra Cortical Facilitation; sMRI/fMRI, structural and functional Magnetic Resonance Imaging; TMS, Transcranial Magnetic Stimulation; TS, Test Stimulus.

TABLE 1 | Group data.

	MSP $n = 12$		HC $n = 12$		
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	
Age (years)	39 ± 9	(26–52)	35 ± 10	(23–52)	
Gender (male:female)	6:6		5:7		
EDSS score (median)	2.3		NA		
Disease duration (years)	6 ± 4	1–14	NA		
FSMC total score	41 ± 16	(11–62)	27 ± 8	(20–46)	<0.01
FSMC motor score	27 ± 12	(10–45)	12	(10–23)	<0.01
FSMC cognitive score	31 ± 12	(10–46)	14	(10–24)	<0.01
PASAT score	59 ± 2.4	(51–60)	60	(60)	n.s
SDMT score	0.98 ± 0.1	(0.98–1)	(0.99 ± 0.005)	(0.98–1)	n.s
RMT (Resting Motor Threshold)	68 ± 9	(47–83)	66 ± 12	(44–90)	

The group characteristics of both groups (MS, Multiple Sclerosis; HC, Healthy Controls), displaying the mean and standard derivation (SD) as well as the range of basic demographic and clinical measures, where appropriate. NA, not applicable.

task, premotor-motor connectivity showed a trial-dependent divergence during the late RT period: Go trials led to a dynamic increase, while No Go trails resulted in a dynamic decrease (23).

Building on the work by Groppa et al. (23, 24), we used a slightly modified dsTMS paradigm to examine how movement preparation and movement inhibition dynamically modulate corticospinal excitability and ipsilateral PMd-to-M1 connectivity during the RT period in patients with RR-MS and healthy participants. We also explored whether dynamic changes in functional PMd-to-M1 connectivity during movement initiation or inhibition would scale with subjectively experienced fatigue in MS patients. Since we were interested in the control of dexterous movements, we selected a visually cued pinching task rather than a cued two-choice movement task. We hypothesized that multiple sclerosis would reduce the dynamic modulation of premotor-to-motor facilitation during the RT period and that deficient modulation of premotor-to-motor facilitation in the pre-movement phase would scale with the individual experience of motor fatigue in MS patients.

MATERIALS AND METHODS

Subjects

Fourteen healthy controls (HC) (six men, aged 37.5 ± 10.8 years, mean ± SD) and 14 patients with relapsing-remitting MS (RR-MS) (seven men, aged 37.6 ± 8.5 years, mean ± SD) were enrolled in the TMS study. All subjects were right-handed according to the Edinburgh Handedness Inventory (25) and gave informed consent before participation. Exclusion criteria for participation were (1) drug or alcohol addiction, (2) tiredness as a pharmaceutical side effect, (3) diagnosis of a comorbid neuropsychiatric disorder, and (4) any contraindication to receiving TMS as listed in the guidelines of the International Federation of Clinical Neurophysiology (26). Four participants (two patients and two healthy subjects) had to be excluded because their motor threshold was too high to be stimulated using the small coils; hence, the data of 12 patients with RR-MS and 12 healthy controls were analyzed. The group data of the patients are listed in **Table 1**. The study was approved by the Regional Committee on Health Research Ethics of the Capital Region in Denmark.

All MS patients were recruited from the Danish Multiple Sclerosis Centre (DMSC) at Rigshospitalet in Copenhagen. Patients with a relapsing-remitting disease course, without clinical or radiological relapses for at least 3 months and Expanded Disability Status Scale (EDSS) scores of $\leq$3.5 were selected for the study. Fatigue was assessed with the Fatigue Scale for Motor and Cognitive Functions (FSMC) (27). Cognitive functioning was assessed using the symbol digit modality test (SDMT) and the Paced Auditory Serial Addition Task (PASAT) (28, 29). Participants included in this study were part of a larger multimodal neuroimaging project conducted at the Danish Research Centre for Magnetic Resonance (DRCMR). While all MS patients who participated in the neuroimaging project were offered the opportunity to take part in the additional TMS testing day, only 14 consented to taking part in this TMS sub-study. This low number was likely due to the extensive test protocol that all participants had already undergone prior to the TMS experiment. All participants had completed three testing days, including clinical assessments, structural and functional magnetic resonance imaging, and electroencephalography. These data are reported elsewhere (16).

Experimental Procedure

The experimental procedures are illustrated in **Figure 1**. At the beginning of the experiment, short-interval intracortical facilitation (SICF) at ISIs between 1.0 and 2.0 ms was used to probe the intracortical facilitatory circuits that generate indirect waves (I-waves). This was primarily done to determine the optimal inter-stimulus interval (ISI) for the subsequent main experiment (dsTMS) (23, 24). The individual interval that elicited the strongest SICF was chosen as individual ISI and ensured that the timing between the CS and the TS was set so that that the first I-wave elicited by the TS over M1 coincided with the CS over the PMd [(see (23, 24)]. The intervals that elicit the strongest ipsilateral PMd-M1 facilitation suggest that the ipsilateral PMd-M1-HAND paradigm targets I-wave circuits:

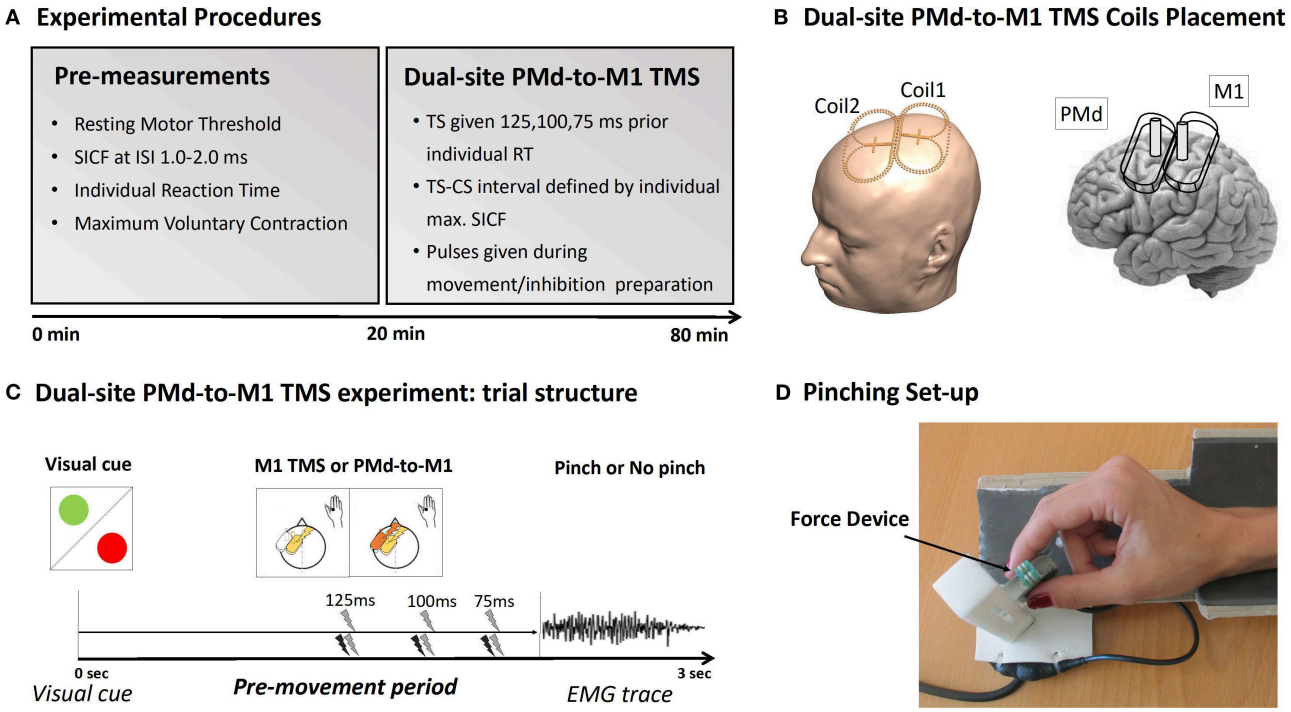


FIGURE 1 | Experimental procedure. **(A)** Description of the experimental TMS procedure. **(B)** Placement of the mini-coils used during the experiment. **(C)** Plot of the Pinch-NoPinch experiment: subjects were instructed by a visual cue to either perform a pinch movement (green circle) or to refrain from preforming the movement (red circle). Single-site TMS over M1 and dual-site TMS over PMd-M1 were given 125, 100, or 75 ms before movement onset. **(D)** The pinch grip device used during the experiment.

Pmd-M1facilitation peaks at around 1.2, 2.4, and 4.0 ms and thereby closely mirrors the three I-wave peaks observed during short intracortical facilitation (SICF).

We also determined the individual mean reaction time during the Pinch-No Pinch task to adjust the timing of the TS during the main experiment. In the main experiment, corticospinal excitability and PMd-to-M1 connectivity were assessed during a visually cued Pinch-NoPinch task. The task was performed with the tips of the thumb and index finger of the right hand. Single- and dual-site TMS trials were intermixed, allowing the influence of action context on both corticomotor excitability and PMd-to-M1 connectivity to be tested in the same experiment. It is important to stress that the Pinch-NoPinch task used in this experiment is different from a classical Go-NoGo task since the pinch task requires a slight isometric force even in the NoPinch condition to keep the sensor in place. Hence the NoPinch condition may not reflect a complete inhibition but instead "downscaling" of pinch force while maintaining the current pinch position.

Experimental Setup

During the experiment, the participants sat in a comfortable chair with an arm- and headrest. While determining the SICF, the arms were placed on the armrest, entirely at rest. During the main experiment, the participants also had their arm on the armrest but held a force device between their right index finger and thumb. The Pinch-NoPinch task required the participants to react to a green or red circle. The color of the circle indicated a pinch (green) or NoPinch (red) trial. Participants had to react to the visual cue by either increasing pinch force or by keeping the force sensor in their hand without increasing pinch force. Before the task, participants were instructed to press the device quickly and with maximal force every time the green circle appeared. This was done to determine the individual reaction time and force level. Throughout the experiment, visual feedback reflecting the applied force was given.

To quantify MEPs both during SICF and the main experiment, the electromyographic (EMG) activity of the right first dorsal interosseus (FDI) muscle was recorded using surface Ag/AgCl electrodes in a bipolar montage. The signal was amplified by the factor 1,000 (D360, Digitimer, Hertfordshire, UK), band-pass filtered between 2 and 2,000 Hz and digitized at a frequency of 5,000 Hz (CED Micro 1401, Cambridge Electronic Design, Cambridge, UK). Signal 4 was used for data acquisition and further analysis (Signal Version 4 for Windows, Cambridge Electronic Design, Cambridge, UK).

Determination of Individual I-Wave Peak and Pinch Reaction Time

SICF

Since I-wave latencies and I-wave facilitation display considerable inter-individual variability (23), we chose to probe PMd-to-M1

connectivity at the inter-pulse interval reflecting the individual I_1-wave peak in each participant. The individual I_1-wave peak was determined using a SICF protocol. The intensity of the TS was set to induce MEPs of about 1 mV in the relaxed right FDI muscle, while the intensity of the CS was set at 90% of Resting Motor Threshold (RMT). Six different inter-stimulus intervals (ISIs) ranging from 1.0 to 2.0 ms (steps of 0.2 ms) were repeated 10 times in pseudorandom order. Trials were averaged for each ISI, and the ISI with the greatest facilitation was used in the subsequent main experiment as ISI between M1-HAND and PMd stimulation. The SICF-curve was measured using an MC-B70 coil connected to a MagPro stimulator (MagVenture, Farum, Denmark). Note that we used a different coil from the ones used for the dual site-TMS session since the small Mag&More coils required for ipsilateral PMD-M1 stimulation did not allow two consecutive pulses to be fired through the same coil at the short inter-pulse intervals required by the SICF. The RMT for both coil types was determined in the relaxed FDI muscle using the Parameter Estimation in Sequential Test (PEST) method (30, 31).

Pinch-Task Reaction Times

To ensure that stimulation in the ds-TMS experiment was given at comparable time points during movement preparation, the individual Reaction Time (RT) for the Pinch trials of the Pinch-NoPinch task was measured before the main experiment. RT was defined as the time point at which participants started to increase the force of their contraction 10% above baseline toward the target. TMS during the main experiment was timed 125, 100, or 75 ms before the individual averaged response time.

The Maximum Voluntary Contraction (MVC) was also calculated for each participant in order to set the individual force level required during the Pinch-NoPinch task. RT and MVC were calculated from averaging 25 Pinch-NoPinch trials without TMS.

Main Experiment

During the main experiment, two mini-coils (56 × 104 mm²) connected to a PowerMAG Research 100 stimulator (Mag&More GmbH, Munich, Germany) were used. The M1-HAND pulse was set to produce an MEP of 0.5 mV amplitude. This intensity was chosen because the small coils were not strong enough to reliably elicit an MEP of 1 mV. The intensity was determined using the PEST method (30, 31) while the hand was already in position for the pinch task. The M1 coil was placed tangentially to the scalp at a 45° angle over the functional hotspot for the right FDI. The intensity for the PMd coil was set to 90% of the RMT, and the location of the PMd coil was determined by physically attaching it to the M1 coil (24). The TS to M1 was given 125, 100, or 75 ms prior to individual movement onset (24). In half of all trials, a conditioning pulse to PMd followed the test pulse. The ISI between both pulses was set to the individual I_1-wave peak determined by the SICF. Hence, the experiment tested corticospinal excitability (TS-only trials) and PMd-M1 connectivity (PMd-M1 pairs) in six different conditions (Action Context: Pinch/NoPinch; Action Timing: 125, 100, 75 ms prior to movement onset). Trials were pseudo-randomly intermixed, and, for each condition, 12 MEPs were recorded, leading to 144 trials per subject. Neuronavigation (TMS Navigator, LOCALITE, St. Augustine, Germany) of the M1 coil (and, by proxy, of the physically attached PMd coil) allowed precise monitoring of the coil position throughout the experiment.

Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics (version 22 for Windows) with the significance threshold set at $P < 0.05$. The Kolmogorov–Smirnov test and Mauchly test were performed to verify the assumptions of normality and sphericity in the distribution of all the data. The Greenhouse-Geisser correction method was used to correct for non-sphericity. Group matching regarding age, gender, and RMT were tested with independent sample *t*-test and χ^2 depending on the data type. Bonferroni correction was applied to correct for multiple comparisons. Data are given as mean ± standard error of the mean (SEM).

Short Latency Intracortical Facilitation

All individuals showed their peak facilitation between 1.2 and 1.6 ms. While the SICF was primarily done to individualize the ISI in the main experiment, intracortical facilitation was also tested for group differences, focusing on the ISI at which the SICF peaked in each subject. MEP amplitudes (normalized to the test stimulus) at the peak ISIs were tested for group differences using an independent sample *t*-test.

Behavioral Data

To evaluate the difference in Reaction Time (RT) during the Pinch-NoPinch task, we used a mixed-effects model ANOVA with *Group* (two levels: MS and HC) as the between-subject factor and *TMS* (two levels: TS-only/TS-CS) and *Time* (three levels: 75/100/125 ms prior to average RT) as within-subject factors.

Corticospinal Excitability During Pinch-NoPinch Task

To investigate corticospinal excitability during a Pinch-NoPinch task, all TS-only trials were analyzed in a mixed-effects ANOVA using the normalized MEPs as the dependent variable, with *Group* (two levels: RR-MS and HC) as the between-subject factor and *Task* (two levels: Pinch/NoPinch) and *Time* (three levels: 75/100/125 ms prior to average RT) as within-subject factors. *Post-hoc* tests following significant main effects or interaction effects were Bonferroni-corrected.

PMd-M1 Connectivity During Pinch-NoPinch Task

To investigate ipsilateral PMd-M1 connectivity, all TS-CS trials were analyzed using a mixed-effects ANOVA with *Group* (two levels: MSP/HC) as the between-subject factor and *Task* (two levels: Pinch-NoPinch) and *Time* (three levels: 75/100/125 ms prior to average RT) as within-subject factors. MEPs were normalized to the single pulses given at the same Time and Task.

Correlations

To test whether abnormal PMd-M1 connectivity in patients correlates with fatigue severity (motor score), we performed Pearson correlation analysis. Since we expected task-dependent modulation of PMd-M1 connectivity to increase closer to movement initiation or inhibition, we calculated the change in normalized PMd-conditioned MEP-size when approaching

TABLE 2 | TMS group data.

	MSP $n = 12$			HC $n = 12$		
	Mean	**SD**	**Range**	**Mean**	**SD**	**Range**
SICF						
ISI	1.2			1.2		
RMT	34.67	4.60	28–43	34.42	6.27	(24–47)
dsTMS						
RT (ms)	359.60	41.50	(274.4–433.2)	397.94	77.48	(301.2–526.3)

Basic neurophysiologic measures of both groups (MS = Multiple Sclerosis; HC = Healthy Controls), displaying the mean and standard derivation (SD) as well as the range of basic demographic and clinical measures, where appropriate. ISI (interstimulus interval) denotes the most effective inter-stimulus interval during the SICF (Short Intracortical Facilitation) protocol and RMT the average Resting Motor Threshold. RT denotes the reaction time to initiate a pinch force during the Go condition of the PinchNoPinch task.

Pinch and NoPinch execution (Δ 100–75 ms) and correlated these values with individual FCMS severity scores. To test for the timing specificity of our results, we also calculated correlations between PMd-conditioned MEPs at a different delta interval (Δ 125–100 ms). Bonferroni correction was used to obtain family-wise error-corrected p-values where appropriate.

RESULTS

Basic Group Characteristics

Age and sex were not significantly different between healthy controls and patients (age: $t(22) = -0.854$, $p = 0.402$; sex: $\chi_{(1)} = 0.168$, $p = 0.682$). Neither were cognitive test scores (PASAT & SDMT), basic neurophysiological parameters like RMT, or the stimulator output used to achieve 1 mV during SICF or 0.5 mv during the main experiment (all $p > 0.40$).

Short Latency Intracortical Facilitation

Test stimulus size during the SICF was not significantly different between groups ($t(21) = -1.616$; $p = 0.12$). All participants did show SICF in at least one interval, and all participants had their most responsive interval (e.g., the interval with the highest facilitation) between 1.2 and 1.6 ms (**Table 2**). To test for differences in intracortical facilitation between groups, the MEP amplitude for the most effective SICF interval was normalized to the test pulse in each participant. A *t*-test comparing the optimal SICF between groups showed that RR-MS patients showed significantly less facilitation than healthy volunteers ($t(22) = 2.39$; $p = 0.026$) (**Figure 2**).

Behavioral Data

We tested whether either group membership (RR-MS vs. HC), TMS condition (TS vs. TS-CS), or TMS timing (75/100/125 ms) influenced the performance during the Pinch-NoPinch task. A mixed-effects ANOVA with RT as the dependent variable showed that none of the factors had a significant influence on RT (Time: $F_{(1.073, 23.597)} = 1.458$, $p = 0.242$; Group ($F_{(1, 22)} = 1.454$, $p = 0.241$; Condition: $F_{(1, 22)} = 0.499$, $p = 0.487$). Also, none of the interactions were significant ($p > 0.2$).

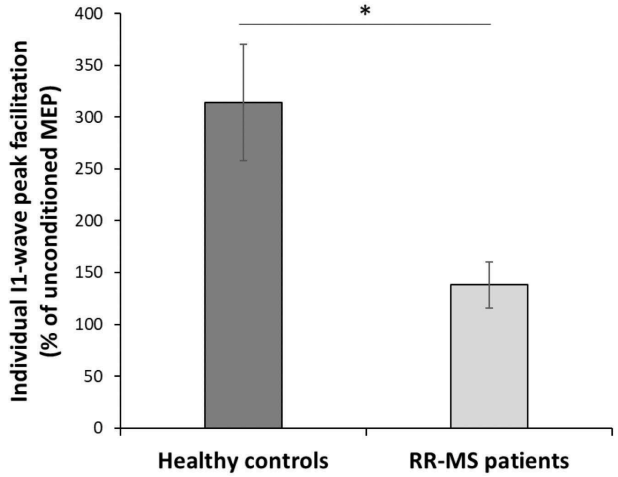


FIGURE 2 | Short Intracortical Facilitation (SICF). Individual most effective ISIs normalized with respect to the TS. Multiple sclerosis patients show a significantly lower amplitude of the I1-wave peak facilitation with respect to the HC ($t(22) = 2.396$; $p = 0.026$); *$p < 0.05$.

Single-Pulse TMS Data

To test for action context-dependent modulations of corticospinal excitability, a mixed-effects ANOVA was calculated, with the MEPs elicited by the TS during the Pinch-NoPinch task as the dependent variable. The ANOVA indicated that the action context modulated corticospinal excitability in both groups, with higher MEPs during the Pinch-trials (main effect of Task: $F_{(1, 22)} = 7.068$, $p = 0.014$). Mean MEP amplitude evoked by TS in the Pinch and NoPinch conditions was consistently larger than the 0.5 mV MEP amplitude evoked by the TS at rest, as determined before the start of the experiment. A significant Time × Task interaction further indicated that corticospinal facilitation increased when the TS was given closer to Pinch onset and dropped (though not under baseline) when the TS was given closer to the (imaginary) in the No-Pinch condition (**Figure 3A**). ($F_{(2, 44)} = 4.123$, $p = 0.023$). The lack of a significant *Group* effect indicated that there was no significant difference in time-dependent modulation of corticospinal excitability between the groups (**Figures 3B,C**). This indicated that MS patients modulated corticospinal excitability in a task-dependent fashion and, to a degree, were comparable to healthy controls ($F_{(1, 22)} = 0.320$, $p = 0.578$) (**Figure 3C**).

Dual-Site TMS Data: Modulations of Premotor-M1 Connectivity

A mixed-effects ANOVA calculated on the normalized MEPs elicited by dual-site TMS did not reveal a significant effect of *Group*, *Task*, or *Time* on PMd-M1 connectivity. However, there was a trend toward a *Time* × *Group* interaction ($F_{(2, 44)} = 2.760$, $p = 0.074$; **Figure 4A**). *Post-hoc* tests did not find significant group differences but indicated that the difference between groups was largest closest to movement onset (75 ms, $p = 0.1$) (**Figures 4A–C**).

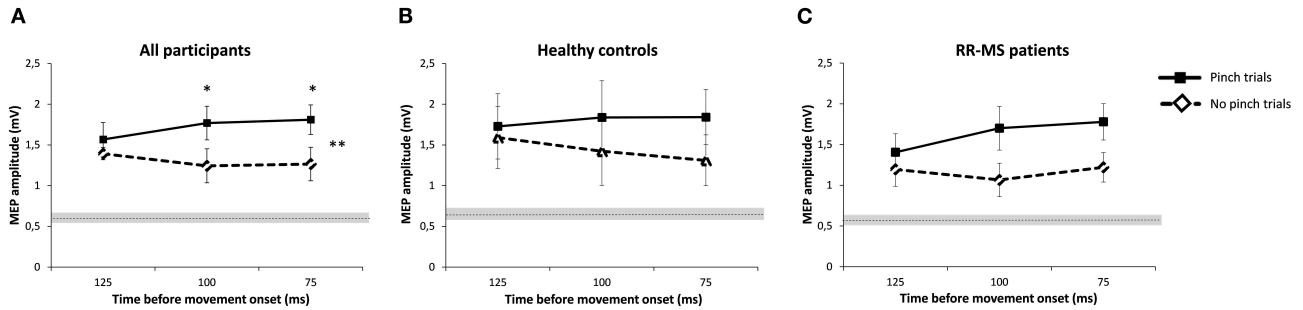


FIGURE 3 | Pinch-NoPinch task. **(A)** Pre-movement facilitation of the MEP amplitude during the Pinch/NoPinch task, averaged over all participants. The dotted, horizontal line indicates the mean value of the baseline MEP (0.63 mV) with standard error as a gray shadow (0.06). Error bars indicate standard error. **(B)** Mean MEP amplitudes of the HC group during the task. The dotted horizontal line indicates the mean value of the baseline MEP for HC (0.65 mV± 0.09). Error bars indicate standard error. **(C)** Mean MEP amplitudes of the MS group during the task. The dotted horizontal line indicates the mean value of the baseline MEP for MS patients (0.61 mV ± 0.09). Error bars indicate standard error. *$p < 0.05$.

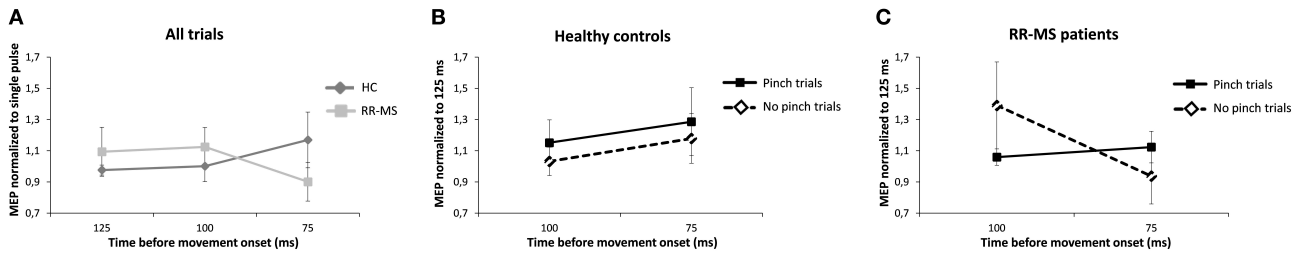


FIGURE 4 | Dual-site TMS (dsTMS) protocol during the Pinch-NoPinch Task. Pre-movement MEP amplitude modulation during the Pinch-NoPinch task. Each line represents the mean amplitude of the MEPs elicited by dsTMS, normalized to the mean MEP amplitude elicited by the single pulse at the same time interval. Error bars indicate standard error. **(A)** Modulation of MEP amplitude between HC and MS patients over time. **(B)** Change in PMd-M1 modulation when getting closer to (potential) movement onset for the HC-group, normalized to the mean MEP at 125 ms. **(C)** Change in PMd-M1 modulation when getting closer to (potential) movement onset for the MS-group normalized to the mean MEP at 125 ms.

Dynamic Modulation of PMd-M1 Connectivity as a Marker of Motor Fatigue

In patients, we found a significant positive correlation between increasing PMd-M1 inhibition, indicated by ΔMEP (100–75 ms), and the FSMC motor score for the NoPinch trials but not for the Pinch task (NoPinch: $r = 0.69$, $p = 0.007$; Bonferroni-corrected $p = 0.028$; Pinch: $r = 0.21$, $p = 0.50$; **Figure 5**). While a similar trend could be observed for ΔMEP (125–100 ms), NoPinch correlations for this interval were weaker and did not survive Bonferroni correction (NoPinch: $r = 0.587$; $p = 0.022$; Bonferroni-corrected $p = 0.08$; Pinch $r = -0.175$; $p = 0.293$). In healthy subjects, no significant correlations could be observed ($p > 0.2$). There was also no correlation between the dynamic modulation of corticospinal excitability as revealed by single-pulse TMS and individual FSMC scores.

DISCUSSION

Using single-pulse and paired-pulse TMS, we studied the dynamic modulation of motor cortical excitability during a cued Pinch-NoPinch task in patients with RR-MS. Our measurements yielded two main findings: First, patients showed a normal modulation of corticospinal excitability as well as ipsilateral premotor-to-motor cortical drive during the RT period of Pinch and NoPinch trials. Second, patients showed a tendency toward attenuation of the premotor-to-motor drive toward the end of the RT period in NoPinch trials. This attenuation in functional premotor-to-motor connectivity scaled positively with the amount of motor fatigue in patients. Mean response times did not differ between groups or TMS conditions, indicating that our TMS measurements during the RT period were not confounded by relative differences in the timing of TMS with respect to the appearance of the cue. The comparable RTs also exclude the possibility that TMS prolonged the RT period in patients with MS relative to healthy controls (32). Importantly, measures of cognitive functioning did not differ between groups indicating that executive control processes like information processing efficiency and speed were not affected in the tested patients (33).

Modulation of Corticospinal Excitability During the RT Period

Single-pulse TMS applied to the left M1-HAND revealed a comparable modulation of corticospinal excitability in patients with RR-MS and healthy controls. Both groups showed a substantial increase in corticospinal excitability across all ISIs and experimental conditions. This indicates that the RT period was characterized by "global" corticospinal facilitation. It also implies that the decision to refrain from further action in NoPinch trials

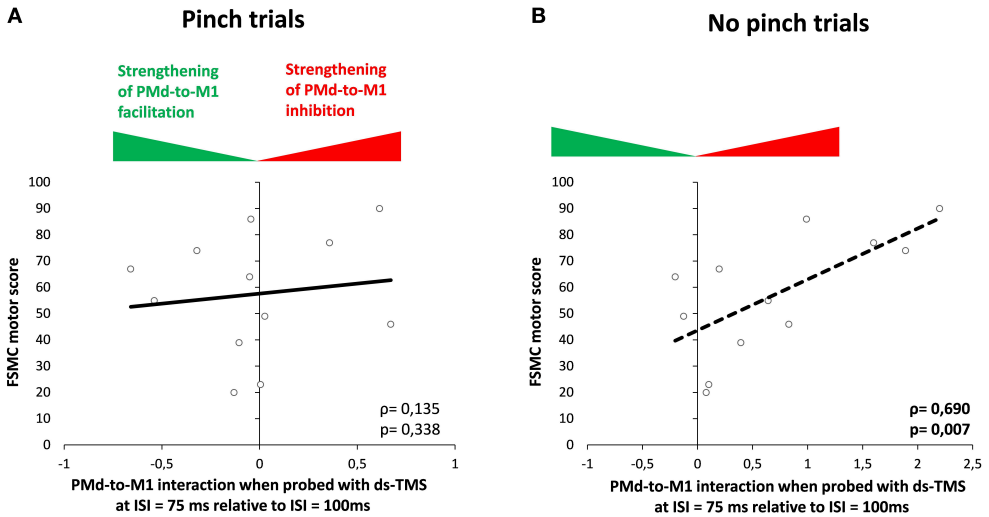


FIGURE 5 | Correlation analysis. Correlation between FSMC motor score and the normalized modulation of MEP from 100 to 75 ms (Δ score) during the Pinch **(A)** and NoPinch **(B)** tasks in the MS group. M1, primary motor cortex; PMd, dorsal premotor cortex.

did not require global inhibition but rather a gradual downscaling of facilitation.

In addition to global MEP facilitation, there was a time-dependent modulation of corticospinal excitability during the RT period, caused by a divergence of corticospinal excitability in Pinch and NoPinch trials toward the end of the RT period. Corticospinal excitability increased further if the visual cue instructed participants to pinch. Conversely, corticospinal excitability showed a relative decrease during the RT period if the visual cue required participants to refrain from pinching. This finding agrees with previous studies demonstrating context-dependent regulation of excitability using a classical Go-NoGo task (34, 35), even though the classical NoGo condition leads to active suppression of corticospinal excitability rather than the relative downscaling of facilitation observed during NoPinch trials in our study.

The differential effect of action context on the dynamic regulation of corticospinal excitability was similar in patients with MS and healthy controls. This observation is in contrast with previous single-pulse TMS studies, which found attenuated pre-movement facilitation of MEP amplitude in simple cued RT tasks (14, 15). The discrepant findings highlight that the specific movement context may be pivotal in detecting disease-dependent changes in pre-movement excitability. Indeed, differences in the motor context can reconcile the apparently diverging findings. The simple, cued RT-task used in previous studies required a rapid initiation and release of the same action across all trials based on a very simple cue-response mapping rule. In contrast, cue-response mapping was more complex in the Pinch-NoPinch RT task used in the present study and required fine control of grip force levels during pinching. We argue, therefore, that a disease-dependent reduction of pre-movement facilitation does not generalize across motor tasks. Rapid boosting of corticospinal excitability during simple externally cued motor actions appears to be impaired in MS and associated with motor fatigue (14, 15). In contrast, more finely tuned and bi-directional regulation of corticospinal excitability during deliberate choices to act (to pinch) or not to act (not to pinch) may be unaffected—at least in moderately affected patients with RR-MS. Another factor that may have helped patients to reach standard pre-movement modulation was the overall corticomotor facilitation induced by the task: the substantial increase in corticospinal excitability throughout all ISIs and experimental conditions indicates a "global" corticospinal facilitation and suggests that the decision to refrain from further action in NoPinch trials did not require global inhibition but rather a downscaling of facilitation.

Ipsilateral Premotor-to-Motor Drive

Our dsTMS measurements revealed no differences in the ipsilateral premotor-to-motor drive between patients with RR-MS and healthy controls. In both groups, the CS given to the left PMd elicited no extra facilitation of the MEPs evoked with a TS over ipsilateral M1-Hand. This was the case when participants were about to initialize a pinch or refrained from executing a pinch. The results suggest that ipsilateral premotor-motor drive may have already been saturated during the RT period, which may have prevented the premotor CS from further increasing premotor-to-motor facilitation.

The dsTMS results show that the relative strength of effective ipsilateral PMd-to-M1-HAND facilitation was constant throughout the task and was not consistently altered by MS. However, subtle alterations in premotor-to-motor connectivity were observed in patients with RR-MS at the last ISI, which was closest to the end of the RT period. While healthy controls displayed a non-significant faciliatory premotor-motor effect in both trial conditions, MS patients showed a slightly inhibitory influence of the ipsilateral premotor CS on corticospinal excitability during NoPinch trials. Correlation analysis demonstrated that the individual magnitude of premotor-to-motor inhibition scaled linearly with the severity of motor

fatigue in MS patients. The more severe the motor fatigue, the stronger the inhibitory drive from ipsilateral PMd to M1-HAND was approaching a NoPinch event. This relationship was not present in the Pinch trials and was not found in the healthy control group. This finding indicates that patients who are more affected by motor fatigue show stronger downregulation of effective PMd-to-M1 connectivity in a context of movement inhibition and this may indicate a less effective mode of fine tuning the PMd-to-M1 drive.

Short-Latency Intracortical Facilitation

Using paired-pulse TMS applied to the precentral gyrus, we replicated a previous paired-pulse TMS study showing a relative reduction in SICF in MS patients relative to healthy controls (9). However, unlike the previous study, we were not able to observe a correlation between individual reduction in SICF and disease severity (9).

Caveats

The presented study was subsidiary to a larger project (16), and hence the number of participants was determined by the retention rate at which participants agreed to participate in the additional testing day. While previous studies on the action context-dependent modulation of premotor-motor connectivity (24) have used comparable sample sizes, it is possible that the sample of 12 participants per group reduced the chance of detecting subtle between-group differences. Future studies are needed to determine whether the reported trend for a general *Time* × *Group* interaction in premotor-M1 connectivity would become significant in a better-powered study.

CONCLUSIONS

Our data suggest that pre-movement facilitation in M1 is not impaired in MS patients if probed in a complex context of action preparation and action inhibition. However, we were able to demonstrate subtle abnormalities in premotor-motor connectivity, where decreasing PMd-M1HAND facilitation during movement inhibition predicted the severity of fatigue scores in MS patients. This may indicate that patients suffering from motor fatigue require stronger modulation of their PMd-M1 drive to implement movement inhibition. Our findings challenge disease-dependent modulation of corticospinal excitability and indicate that functional premotor-motor connectivity may be important in understanding the pathology of fatigue in MS.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Committee on Health Research Ethics of the Capital Region in Denmark. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ERu, RD, ERa, AK, and HS designed the study. ERu, OS, and AK collected the data. ERu, RD, KM, and KA analyzed the data. ERu, ERa, RD, AK, KM, and HS wrote the paper.

FUNDING

This study was supported by the Danish Multiple Sclerosis Society (Scleroseforeningen) (Grant number: R308-A19386-B2785, R367-A25015-B2785, R399-A27956-B2785, and R431-A29804-B2785) and the Lundbeck Foundation (ContAct) (Grant number: R59-A5399). HS holds a 5-year professorship in precision medicine at the Faculty of Health Sciences and Medicine, University of Copenhagen which is sponsored by the Lundbeck Foundation (Grant number: R186-2015-2138).

ACKNOWLEDGMENTS

We thank Meaghan Elisabeth Spedden for proofreading and help with editing.

REFERENCES

1. Berer K, Krishnamoorthy G. Microbial view of central nervous system autoimmunity. *FEBS letters*. 2014;588(22):4207–13.
2. Calabrese M, Gallo P, Calabrese M, Filippi M, Gallo P. Cortical lesions in multiple sclerosis. *Nat Publ Group*. (2010) 6:438–44. doi: 10.1038/nrneurol.2010.93
3. Matza LS, Kim K, Phillips G, Zorn K, Chan KS, Smith KC, et al. Multiple sclerosis relapse: qualitative findings from clinician and patient interviews. *Mult Scler Relat Disord*. (2019) 27:139–46. doi: 10.1016/j.msard.2018.09.029
4. Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N Committee. *Clin Neurophysiol*. (2015) 126:1071–107. doi: 10.1016/j.clinph.2015.02.001
5. Snow NJ, Wadden KP, Chaves AR, Ploughman M. Transcranial magnetic stimulation as a potential biomarker in multiple sclerosis: a systematic review with recommendations for future research. *Neural Plasticity*. (2019) 2019:6430596. doi: 10.1155/2019/6430596
6. Neva JL, Lakhani B, Brown KE, Wadden KP, Mang CS, Ledwell NH, et al. Multiple measures of corticospinal excitability are associated with clinical features of multiple sclerosis. *Behav Brain Res*. (2016) 297:187–95. doi: 10.1016/j.bbr.2015.10.015
7. Thickbroom GW, Byrnes ML, Archer SA, Kermode AG, Mastaglia FL. Corticomotor organisation and motor function in multiple sclerosis. *J Neurol*. (2005) 252:765–71. doi: 10.1007/s00415-005-0728-9
8. Conte A, Lenzi D, Frasca V, Gilio F, Giacomelli E, Gabriele M, et al. Intracortical excitability in patients with relapsing-remitting and secondary progressive multiple sclerosis. *J Neurol*. (2009) 256:933–8. doi: 10.1007/s00415-009-5047-0

9. Mori F, Kusayanagi H, Monteleone F, Moscatelli A, Nicoletti CG, Bernardi G, et al. Short interval intracortical facilitation correlates with the degree of disability in multiple sclerosis. *Brain Stimul.* (2013) 6:67–71. doi: 10.1016/j.brs.2012.02.001
10. Vucic S, Burke T, Lenton K, Ramanathan S, Gomes L, Yannikas C, et al. Cortical dysfunction underlies disability in multiple sclerosis. *Mult Scler.* (2012) 18:425–32. doi: 10.1177/1352458511424308
11. Disanto G, Berlanga AJ, Handel AE, Para AE, Burrell AM, Fries A, et al. Heterogeneity in multiple sclerosis: scratching the surface of a complex disease. *Autoimmune Dis.* (2010) 2011:932351. doi: 10.4061/2011/932351
12. Severijns D, Zijdewind I, Dalgas U, Lamers I, Lismont C, Feys P. The assessment of motor fatigability in persons with multiple sclerosis: a systematic review. *Neurorehabil Neural Repair.* (2017) 31:413–31. doi: 10.1177/1545968317690831
13. Liepert J, Mingers D, Heesen C, Baumer T, Weiller C. Motor cortex excitability and fatigue in multiple sclerosis: a transcranial magnetic stimulation study. *Mult Scler.* (2005) 11:316–21. doi: 10.1191/1352458505ms1163oa
14. Russo M, Crupi D, Naro A, Avanzino L, Buccafusca M, Dattola V, et al. Fatigue in patients with multiple sclerosis: from movement preparation to motor execution. *J Neurol Sci.* (2015) 351:52–7. doi: 10.1016/j.jns.2015.02.031
15. Morgante F, Dattola V, Crupi D, Russo M, Rizzo V, Ghilardi MF, et al. Is central fatigue in multiple sclerosis a disorder of movement preparation? *J Neurol.* (2011) 258:263–72. doi: 10.1007/s00415-010-5742-x
16. Svolgaard O, Andersen KW, Bauer C, Madsen KH, Blinkenberg M, Selleberg F, et al. Cerebellar and premotor activity during a non-fatiguing grip task reflects motor fatigue in relapsing-remitting multiple sclerosis. *PLoS ONE.* (2018) 13:e0201162. doi: 10.1371/journal.pone.0201162
17. Bonzano L, Pardini M, Roccatagliata L, Mancardi GL, Bove M. How people with multiple sclerosis cope with a sustained finger motor task: a behavioural and fMRI study. *Behav Brain Res.* (2017) 325(Pt A):63–71. doi: 10.1016/j.bbr.2017.02.008
18. Specogna I, Casagrande F, Lorusso A, Catalan M, Gorian A, Zugna L, et al. Functional MRI during the execution of a motor task in patients with multiple sclerosis and fatigue. *Radiol Med.* (2012) 117:1398–407. doi: 10.1007/s11547-012-0845-3
19. Groppa S. Multifocal TMS for temporo-spatial description of cortico-cortical connectivity patterns. *Clin Neurophysiol.* (2016) 127:1005–6. doi: 10.1016/j.clinph.2015.07.012
20. Ferbert A, Priori A, Rothwell JC, Day BL, Colebatch JG, Marsden CD. Interhemispheric inhibition of the human motor cortex. *J Physiol.* (1992) 453:525–46. doi: 10.1113/jphysiol.1992.sp019243
21. Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I. Magnetic stimulation over the cerebellum in humans. *Ann Neurol.* (1995) 37:703–13. doi: 10.1002/ana.410370603
22. Karabanov A, Jin SH, Joutsen A, Poston B, Aizen J, Ellenstein A, et al. Timing-dependent modulation of the posterior parietal cortex-primary motor cortex pathway by sensorimotor training. *J Neurophysiol.* (2012) 107:3190–9. doi: 10.1152/jn.01049.2011
23. Groppa S, Schlaak BH, Münchau A, Werner-Petroll N, Dünnweber J, Bäumer T, et al. The human dorsal premotor cortex facilitates the excitability of ipsilateral primary motor cortex via a short latency cortico-cortical route. *Human Brain Mapp.* (2012) 33:419–30. doi: 10.1002/hbm.21221
24. Groppa S, Werner-Petroll N, Münchau A, Deuschl G, Ruschworth MFS, Siebner HR. A novel dual-site transcranial magnetic stimulation paradigm to probe fast facilitatory inputs from ipsilateral dorsal premotor cortex to primary motor cortex. *NeuroImage.* (2012) 62:500–9. doi: 10.1016/j.neuroimage.2012.05.023
25. Oldfield RC. The assessment and analysis of handedness: the edinburgh inventory. *Neuropsychologia.* (1971) 9:97–113. doi: 10.1016/0028-3932(71)90067-4
26. Groppa S, Oliviero A, Eisen A, Quartarone A, Cohen LG, Mall V, et al. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol.* (2012) 123:858–82. doi: 10.1016/j.clinph.2012.01.010
27. Penner IK, Raselli C, Stocklin M, Opwis K, Kappos L, Calabrese P. The Fatigue Scale for Motor and Cognitive Functions (FSMC): validation of a new instrument to assess multiple sclerosis-related fatigue. *Mult Scler.* (2009) 15:1509–17. doi: 10.1177/1352458509348519
28. Rao SM, Leo GJ, Haughton VM, St Aubin-Faubert P, Bernardin L. Correlation of magnetic resonance imaging with neuropsychological testing in multiple sclerosis. *Neurology.* (1989) 39(2 Pt 1):161–6. doi: 10.1212/WNL.39.2.161
29. Benedict RH, DeLuca J, Phillips G, LaRocca N, Hudson LD, Rudick R, et al. Validity of the symbol digit modalities test as a cognition performance outcome measure for multiple sclerosis. *Mult Scler.* (2017) 23:721–33. doi: 10.1177/1352458517690821
30. Borckardt JJ, Nahas Z, Koola J, George MS. Estimating resting motor thresholds in transcranial magnetic stimulation research and practice: a computer simulation evaluation of best methods. *J ECT.* (2006) 22:169–75. doi: 10.1097/01.yct.0000235923.52741.72
31. Karabanov AN, Raffin E, Siebner HR. The resting motor threshold–restless or resting? A repeated threshold hunting technique to track dynamic changes in resting motor threshold. *Brain Stimul.* (2015) 8:1191–4. doi: 10.1016/j.brs.2015.07.001
32. Zeller D, Dang SY, Stefan K, Biller A, Bartsch A, Saur D, et al. Functional role of ipsilateral motor areas in multiple sclerosis. *J Neurol Neurosurg Psychiatry.* (2011) 82:578–83. doi: 10.1136/jnnp.2010.219964
33. Migliore S, Curcio G, Couyoumdjian A, Ghazaryan A, Landi D, Moffa F, et al. Executive functioning in relapsing-remitting multiple sclerosis patients without cognitive impairment: a task-switching protocol. *Mult Scler.* (2018) 24:1328–36. doi: 10.1177/1352458517719149
34. Yamanaka K, Kimura T, Miyazaki M, Kawashima N, Nozaki D, Nakazawa K, et al. Human cortical activities during Go/NoGo tasks with opposite motor control paradigms. *Exp Brain Res.* (2002) 142:301–7. doi: 10.1007/s00221-001-0943-2
35. Filipović SR, Jahanshahi M, Rothwell JC, Filipovic SR, Jahanshahi M, Rothwell JC. Cortical potentials related to the nogo decision. *Exp Brain Res.* (2000) 132:411–5. doi: 10.1007/s002210000349

Conflict of Interest: HS has received honoraria as speaker from Sanofi Genzyme, Denmark, and Novartis, Denmark, as a consultant from Sanofi Genzyme, Denmark, and as senior editor (NeuroImage) from Elsevier Publishers, Amsterdam, The Netherlands. He has received royalties as a book editor from Springer Publishers, Stuttgart, Germany. AK is a shareholder and co-founder of BrainChild Technologies, LLC. OS has received travel funding from Biogen Idec. All conflicts of interest are unrelated to this work.

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

frontiers in Neurology

ORIGINAL RESEARCH
published: 04 June 2020
doi: 10.3389/fneur.2020.00422

Walking Training Enhances Corticospinal Excitability in Progressive Multiple Sclerosis—A Pilot Study

*Arthur R. Chaves[1], Augustine J. Devasahayam[1], Morten Riemenschneider[2], Ryan W. Pretty[1] and Michelle Ploughman[1]**

[1] Recovery and Performance Laboratory, Faculty of Medicine, L. A. Miller Centre, Memorial University of Newfoundland, St. John's, NL, Canada, [2] Section for Sports Science, Department of Public Health, Aarhus University, Aarhus, Denmark

Background: Inflammatory lesions and neurodegeneration lead to motor, cognitive, and sensory impairments in people with multiple sclerosis (MS). Accumulation of disability is at least partially due to diminished capacity for neuroplasticity within the central nervous system. Aerobic exercise is a potentially important intervention to enhance neuroplasticity since it causes upregulation of neurotrophins and enhances corticospinal excitability, which can be probed using single-pulse transcranial magnetic stimulation (TMS). Whether people with progressive MS who have accumulated substantial disability could benefit from walking rehabilitative training to enhance neuroplasticity is not known.

Objective: We aimed to determine whether 10 weeks of task-specific walking training would affect corticospinal excitability over time (pre, post, and 3-month follow-up) among people with progressive MS who required walking aids.

Results: Eight people with progressive MS (seven female; 29–74 years old) with an Expanded Disability Status Scale of 6–6.5 underwent harness-supported treadmill walking training in a temperature controlled room at 16°C (10 weeks; three times/week; 40 min at 40–65% heart rate reserve). After training, there was significantly higher corticospinal excitability in both brain hemispheres, reductions in TMS active motor thresholds, and increases in motor-evoked potential amplitudes and slope of the recruitment curve (REC). Decreased intracortical inhibition (shorter cortical silent period) after training was noted in the hemisphere corresponding to the stronger hand only. These effects were not sustained at follow-up. There was a significant relationship between increases in corticospinal excitability (REC, area under the curve) in the hemisphere corresponding to the stronger hand and lessening of both intensity and impact of fatigue on activities of daily living (Fatigue Severity Scale and Modified Fatigue Impact Scale, respectively).

Conclusion: Our pilot results support that vigorous treadmill training can potentially improve neuroplastic potential and mitigate symptoms of the disease even among people who have accumulated substantial disability due to MS.

Keywords: transcranial magnetic stimulation, neuroplasticity, rehabilitation, exercise, progressive multiple sclerosis, corticospinal excitability, fatigue

OPEN ACCESS

Edited by:
Moussa Antoine Chalah, Hôpitaux Universitaires Henri Mondor, France

Reviewed by:
Mathieu Gruet, Université de Toulon, France
Michele Dileone, Hospital Virgen del Puerto, Spain

****Correspondence:***
Michelle Ploughman
michelle.ploughman@med.mun.ca

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *19 February 2020*
Accepted: *22 April 2020*
Published: *04 June 2020*

Citation:
Chaves AR, Devasahayam AJ, Riemenschneider M, Pretty RW and Ploughman M (2020) Walking Training Enhances Corticospinal Excitability in Progressive Multiple Sclerosis—A Pilot Study. Front. Neurol. 11:422. doi: 10.3389/fneur.2020.00422

INTRODUCTION

Multiple sclerosis (MS) is a chronic neurodegenerative disease that causes structural (i.e., brain lesions and atrophy) and functional (i.e., neuronal connectivity and conduction alterations) central nervous system dysfunction (1). Most people with MS are initially diagnosed with the relapsing–remitting form of the disease (RRMS). RRMS is considered to be the inflammatory phase of MS with unpredictable development of central nervous system lesions that result in physical, sensory, and/or cognitive symptoms (i.e., relapses) (2). About 80% of people diagnosed with RRMS will eventually develop secondary progressive MS (SPMS), which is considered to be less inflammatory and more neurodegenerative (2, 3). As well, ∼10% of people with MS present with primary progressive MS (PPMS), in which there is a steady disease progression from initial diagnosis of MS (2, 3). Several lines of evidence suggest that accumulation of disability in progressive MS is related to diminished capacity for neuroplasticity (2–4). Because most disease-modifying drugs act by reducing neuroinflammation, these same treatments do not seem to be as effective during progressive stages (5). Treatments that provide neuroprotection and enhancement of neuroplasticity to recover function and halt MS progression are highly warranted (6–10).

Animal and human research has shown that exercise enhances neuroplasticity by upregulating neurotrophins that facilitate cerebral gliogenesis, neurogenesis, synaptogenesis, and angiogenesis [for reviews see (11, 12)]. In some neurological conditions, such as Alzheimer's disease (13), stroke (12, 14), and spinal cord injury (15), exercise has also been shown to promote neuroplasticity. In MS, studies have shown that engagement in physical exercise training improves aerobic capacity (16, 17), physical function (e.g., walking capacity) (18), and mitigates physical symptoms (e.g., reduce fatigue, muscle weakness) (17, 19, 20). Recent studies support that a high degree of task practice (e.g., constraint-induced movement therapy) can enhance neuroplasticity in people with progressive MS (21), suggesting that there is continued capacity for plasticity even in later stages of the disease.

In humans, rehabilitation-induced neuroplasticity is typically measured using functional brain imaging (22, 23) and transcranial magnetic stimulation (TMS) (24). TMS generates a brief magnetic field through an insulated coil placed on the participant's scalp that induces neuronal activation of the primary motor cortex resulting in a motor-evoked potential (MEP) traveling through the corticospinal tract (24). Studies using TMS in healthy individuals have shown that exercise training promotes corticospinal excitability changes that are related to enhanced neuroplasticity (25–28). Typical TMS biomarkers that demonstrate exercise training-induced changes in corticospinal excitability include lower motor thresholds (29) and higher input-to-output MEP amplitudes responses (28), which are biomarkers mediated by increased glutamatergic (excitatory) neurotransmission (30). As well, in healthy individuals, exercise training has shown to reduce cortical silent period (CSP) duration (27, 31), an interruption of the electromyographic activity of a sustained muscle contraction after TMS-elicited MEP, suggestive of less activity of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (24, 32).

Excessive GABAergic-mediated intracortical inhibition and lower corticospinal excitability measured with longer CSP and higher motor thresholds and lower input-to-output MEP amplitudes, respectively, are biomarkers of neurological impairment (e.g., stroke and MS) (10, 33–38) and reduced neuroplastic potential (39, 40). In MS, demyelination causes delay of the onset latency of the TMS-elicited MEP (41). Since MEP latency shortening is associated with recovery of physical function after stroke (42) and is faster in physically active individuals (29), in addition to excitatory and inhibitory TMS variables, MEP latency could also be altered by exercise (43). Although evidence from cross-sectional studies suggest a possible link between greater physical fitness and enhanced neuroplasticity in MS (44), no study has investigated the long-term effects of exercise training on neuroplasticity-like mechanisms using TMS, particularly in progressive stages of MS.

The primary aim of the present study was to investigate whether a rehabilitative walking training program induced corticospinal excitability changes related to enhanced neuroplasticity in people with progressive MS with severe MS-related walking disabilities. Since excessive fatigue is among the most disabling symptoms in progressive MS (18) and previous research has demonstrated the link between corticospinal excitability, fatigue (44–46), and fitness levels (44, 47), our secondary aim was to investigate whether exercise training-induced corticospinal excitability changes were associated with changes in physical fitness (cardiorespiratory fitness, body fat) (48) and subjective levels of fatigue (49, 50).

MATERIALS AND METHODS

Experimental Design

This study was part of a feasibility and proof-of-principle interventional study aiming at restoring walking function among patients with MS-related walking disability (51). The data on feasibility and restoration of walking have been reported elsewhere (51). This interventional study (10 weeks, 3×/week exercise training) with TMS assessment pre, post, and 3-month follow-up was approved by the local health ethics board prior to initiation (Health Research Ethics Board, #2019.0225, NCT04066972).

Participants

Ten participants were recruited via referral from neurologists and physiotherapists in the local MS clinic, as well as from an outpatient rehabilitation service discharge database. All participants signed informed consent prior to study inclusion. Recruitment and screening details have been described elsewhere (51). Participants were included if they (1) were diagnosed with progressive MS (SPMS or PPMS), (2) reported no relapses 3 months prior to inclusion, (3) presented with walking impairments (e.g., use of bilateral or unilateral gait aids), (4) had disability level ≥6.0 on the Expanded Disease Status

TABLE 1 | Participants' demographics, body composition, and fitness.

ID	MS Type	MS Severity (EDSS 0–10)	Walking Aid	Age Range (years)	DD (years)	Lean mass (Kg)			VO_{2peak} ($mL.min^{-1}kg^{-1}_{LM}$)			Body Fat %		
						Pre	Post	3-mo	Pre	Post	3-mo	Pre	Post	3-mo
1	PPMS	6.5	Walker	55–60	10	57.22	58.47	59.88	20.05	21.71	19.48	45.6	46.6	46.5
2	SPMS	6.5	Walker	55–60	33	43.26	44.64	–	22.61	20.75	–	44.8	44.5	–
3	PPMS	6.5	Walker	40–45	19	54.99	57.06	57.63	24.79	34.28	29.74	35.1	35.4	34.6
4	SPMS	6.0	Cane	45–50	28	29.47	31.18	33.56	41.84	36.98	36.50	39.1	39.6	36.9
5	SPMS	6.5	Cane	35–40	19	54.31	56.05	54.52	33.31	37.87	41.17	39.1	40.0	37.8
6	SPMS	6.0	Cane	70–75	18	32.87	32.32	33.12	31.61	37.69	41.28	34.4	37.4	33.1
7	PPMS	6.5	Walker	70–75	10	–	–	–	27.31[#]	21.69[#]	18.09[#]	–	–	–
8	SPMS	6.0	Cane	25–30	2	41.74	43.56	42.62	48.28	48.66	48.13	44.7	40.8	39.9

DD, disease duration; EDSS, Expanded Disability Status Scale; MS, Multiple Sclerosis; PPMS, primary progressive MS; SPMS, secondary progressive MS; 3-mo, 3-month follow-up. [#]*Participant 7 declined to undergo Dual Energy X-ray Absorptiometry, and the maximal (peak) volume of oxygen uptake (VO_{2peak} $[mL.min^{-1}Kg^{-1}_{LeanMass(LM)}]$ was calculated by diving this participant's VO_{2peak} ($mL.min^{-1}$) by the LM (kg) of total sample mean. 3-mo, 3-month follow-up.*

Scale (EDSS), (5) were capable of participating in physical exercise [as per Physical Activity Readiness Questionnaire (PAR-Q) screening form (52)], and (6) were eligible to undergo TMS (53) and dual energy X-ray absorptiometry (DEXA) (54) as per screening procedures. Written informed consent was obtained from participants for the publication of any potentially identifiable images or data included in this article.

Two participants dropped out during the intervention (51), reporting not being able to commit to the proposed frequency of exercise sessions (3×/week). Eight participants (seven female) completed the intended exercise training, and pre–post data were collected. One participant (number 2) could not be reached during follow-up assessment. Participant demographics are presented in **Table 1**.

Exercise Intervention

Participants underwent 10 weeks (3×/week) of vigorous treadmill walking exercise training in a temperature-controlled room (16°C) (51). The treadmill was equipped with a harness to prevent falls and to support ≤10% of participants' body weight. The dosage target of the exercise was 40 min (5 min warm-up and cool down) at a moderate-high intensity (40–65% heart rate reserve), which was adjusted throughout the training by increasing the speed and incline of the treadmill and/or reducing body weight support. Manual assistance to advance legs and resting breaks of ≤2 min were provided whenever necessary (51).

Outcome Measures

All outcome measures were assessed before the intervention ($n = 8$), after the 10-week period intervention ($n = 8$) and at 3-month follow-up after the exercise intervention had ended ($n = 7$).

Cardiorespiratory Fitness

Levels of cardiorespiratory fitness were assessed as the peak rate of oxygen uptake (VO_{2peak} expressed in ml O_2 min) during a graded maximal exercise test performed on a recumbent stepper (NuStep, Ann Arbor, MI, USA) as described elsewhere (14, 43, 44, 51, 55). Briefly, participants exercised at a cadence of 80 strides per minute while the equipment resistance level (1–10, beginning at level 3) was increased by one level every 2 min. If exhaustion was not reached at resistance level 10 (maximal NuStep resistance), the cadence was increased by 10 strides per minute every 2 min. Heart rate was continuously monitored during the test (H10, Polar Electro Inc., Kempele, Finland). The maximal and resting heart rate were used to calculate the proposed intensities of the exercise sessions [e.g., intensity target = 60% × (heart $rate_{Max}$ – heart $rate_{Rest}$) + heart $rate_{Rest}$]. Fitness levels were calculated as the absolute VO_{2peak} (ml O_2 min) relative to the total lean body mass (kg) (VO_{2peak} = ml O_2 min^{-1} $kg^{-1}{}_{leanmass}$). The latter has been shown to be a more accurate measure of cardiorespiratory fitness in populations with a high body fat percentage (56).

Body Composition

Participants' total body weight (kg), body fat percentage (%), and lean body mass (kg) were assessed using whole-body dual energy X-ray absorptiometry (Discovery-A Densitometer, Hologic Inc., Bedford, MA, USA). Trained technicians calibrated the system prior to each assessment, and built-in software was used to analyze the data (v.12.6.1:3, Hologic Inc., Bedford, MA, USA).

Total Amount of Workload Performed During the Exercise Sessions

Total amount of workload performed was estimated using standardized equations (48). First, the VO_2 (ml O_2 min^{-1} kg^{-1}) uptake during the exercise was calculated using the equation VO_2 (ml O_2 min^{-1} kg^{-1}) = {resting component (3.5 ml O_2 min^{-1} kg^{-1}) + horizontal component [speed (m/min) × 0.1 ml O_2 kg^{-1} m^{-1}] + vertical component [1.8 ml O_2 kg^{-1} m^{-1} × speed (m min^{-1}) × $incline_{FractionalGrade}$]}; adjustments for treadmill changes in speed and incline throughout the exercise were taken into consideration. The averaged VO_2 (ml O_2 min^{-1} kg^{-1}) was transformed into metabolic equivalents. The kilocalorie (kcal)/minute was calculated using the equation kcal/min = (metabolic equivalents × 3.5 × total body weight in kg)/200. Finally, the total amount of workload performed was calculated by multiplying the kcal/minute by the total time in minutes that the participants exercised. These data were calculated from the

first and the last exercise session participants performed during the exercise training and from the exercise session performed during the follow-up visit.

Levels of Fatigue

The intensity of fatigue perceived by the patients was assessed by the Fatigue Severity Scale (FSS) (49), whereas the impact of fatigue on activities of daily living was measured by the Modified Fatigue Impact Scale (MFIS) (50, 57) [for more details, see (51)].

Transcranial Magnetic Stimulation

Monophasic magnetic pulses were delivered to the right and left brain hemispheres using a BiStim 200^2 stimulator (Magstim Co., Whitland, UK). With participants seated, a coil (70 mm figure-of-eight coil; Magstim Co. Whitland, UK) was positioned tangentially to the scalp with the handle pointing backwards and laterally at an 45° angle from the midline perpendicular to the central sulcus to deliver posterior–anterior directed pulses in the area of the primary motor cortex (58). Electromyographic (EMG) activity and MEPs were collected by surface electrodes (Kendall 200 Coviden, Mansfield, MA, USA) placed on the contralateral first dorsal interosseous hand muscle. Assessing corticospinal excitability on a non-exercised muscle (i.e., FDI rather than leg muscles) was considered important in order to more accurately investigate widespread effects on central nervous system mechanisms involved in brain plasticity (59, 60). A neuronavigation system (Brainsight, Rogue Research Inc., Montreal, QC, Canada) was used to ensure consistency of the coil position (i.e., angle and orientation) on participants' scalp during the TMS assessment. The Montreal Neurological Institute brain template was rendered in the BrainSight software and used as a 3-D stereotaxic template (61). The same system was used to collect EMG muscle activity and record MEPs with its built-in EMG system. The system collects at a sample rate of 3 kHz and uses a 2,500 V/V amplification and a gain of 600 V/V with a bandwidth of 16–550 Hz. Stronger and weaker hands were determined during baseline assessment (pre) by EMG recorded in the FDI muscle while participants performed a pinch grip maximal voluntary contraction (MVC) {mean EMG activity during MVC [stronger vs. weaker hand (mean ± SD)]: 106.07 ± 79.3 μV vs. 51.49 ± 45.12 μV; $Z = -2.34$, $p = 0.018$}. In order to be more precise when differentiating between stronger and weaker sides' brain-to-muscle connectivity (potentially less and more affected sides, respectively), EMG signal was prioritized over force production, since EMG represents the electrical activity from motor units firing action potentials generated by the central nervous system.

Motor thresholds and MEP latency

Suprathreshold TMS stimulations were delivered at different locations around the hand primary motor area. The location with the highest average peak-to-peak MEP amplitude was chosen as the hotspot. The hotspot was reassessed at pre, post, and follow-up, since it can show variability (62) and changes following interventions [e.g., exercise (63)]. The relative frequency method was used to determine resting motor thresholds (RMTs) and active motor thresholds (AMTs) (24, 64) and were determined as the minimum TMS intensity (maximal stimulator output percentage, MSO%) required to elicit peak-to-peak MEP amplitudes of ≥50 μV at rest (RMT) and ≥200 μV with participant performing 10% of pinch grip MVC (AMT) in at least 5 out of 10 trials. RMT and AMT are reported as MSO% (0–100). MEP latencies were determined from the valid MEPs collected during the RMT experiment and were calculated as the time [in milliseconds (ms)] between the TMS artifact and the MEP onset; the timepoint where the MEP amplitude surpassed ±2 standard deviation from the mean EMG background activity (100 ms prior to the TMS stimulation).

Excitatory and inhibitory recruitment curves

To create recruitment curves, TMS stimulation intensities of 105–155% of AMT (increments of 10%) were employed in randomized order with participants performing a pinch grip at 10% of MVC (47). Three to six stimulations (28, 65, 66) were delivered at each intensity, and the averaged peak-to-peak MEP amplitude (μV) and CSP time (ms) were recorded. CSP was defined as the time between the MEP onset to the return of EMG activity (≥±2 standard deviation from background EMG activity) (24). MEP amplitudes were normalized to the largest peak-to-peak amplitude (25) collected during baseline assessment (i.e., first TMS session; prior to beginning of the exercise training). A linear relationship between the normalized MEP amplitudes against the used TMS intensities (105–155% of AMT) determined the excitatory recruitment gain and accuracy (slope and R^2 of the linear relationship, respectively) of the corticospinal tract in recruiting neurons (25, 34), both previously reported potential biomarkers of corticospinal tract integrity (67). Similarly, the inhibitory recruitment curve slope and R^2 was calculated by plotting the CSP time against the TMS intensities. As an estimate of overall corticospinal excitation (MEP amplitudes) and inhibition (CSP time), the area under the curve was calculated using the trapezoid rule $\Delta X \times (Y1 + Y2)/2$, with X being the TMS intensity used (105–155% of AMT) and Y being the normalized MEP amplitudes (% of largest baseline MEP) or the recorded CSP time.

Statistical Analysis

A priori, we planned to use a one-way repeated measures analysis of variance and Friedman test when testing normal and non-normally distributed data, respectively. Because tests of normality (e.g., Shapiro–Wilk) typically require samples sizes of $n \geq 10$ to generate reliable results (68), the more robust non-parametric alternative (i.e., Friedman test) (69) was preferred (70) to determine changes in TMS variables [RMT, AMT, and excitatory and inhibitory recruitment curves (MEP amplitudes$_{105-155\%AMT}$, CSP time$_{105-155\%AMT}$, slope, R^2, and area under the curve)], fitness (ml min^{-1} kg$_{LM}^{-1}$, body fat %), and workload performed (kcal/session), at the different time points (pre, post, and follow-up). Analysis between time points (pre vs. post vs. follow-up) is reported as $\chi^2_{(degreesoffreedom)} =$ test statistic, p-value. When statistically significant ($p < 0.05$), Bonferroni-corrected pairwise comparisons were performed to identify the difference across time points, and the adjusted p-value for multiple comparisons is reported. All data in the text are presented as median (Mdn).

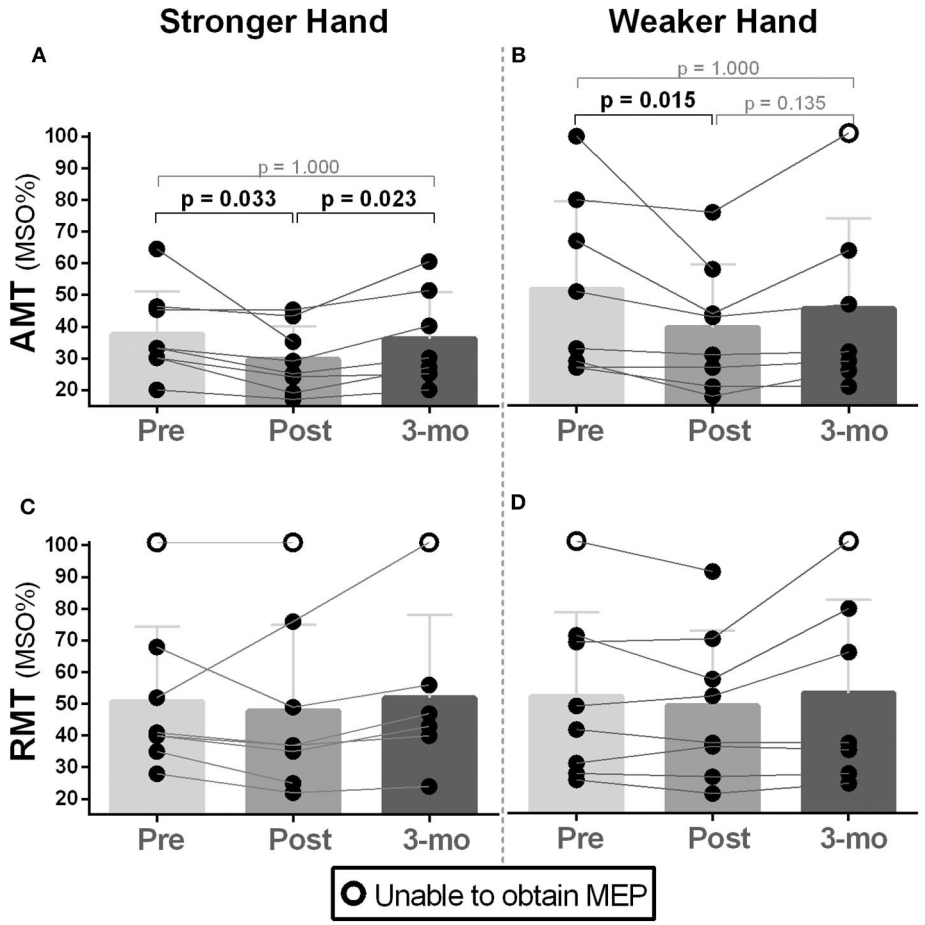


FIGURE 1 | Effects of 10-week treadmill walking exercise training on active and resting motor thresholds. **(A,B)** Increased corticospinal excitability (CSE) was noted during active motor threshold (AMT) assessment in both brain hemispheres (i.e., corresponding to the weaker and stronger hands) as lower values of the maximal stimulator output (MSO%) were needed to elicit motor-evoked potentials (MEPs) in the contralateral first dorsal interosseous muscle (200 μV amplitude MEPs collected during 10% of pincer grip maximal voluntary contraction). AMT returned to baseline during the 3-month follow-up period assessment (3 mo). **(C,D)** There was no difference in MSO% between time points (pre, post, 3-month follow-up) for resting motor threshold (RMT) (i.e., MEPs collected during resting) measured in the hemisphere corresponding to the weaker hand. Because the absence of MEPs is an outcome that represents too low CSE (i.e., 100% of MSO not eliciting MEPs) (71), participants in this condition are represented as open circles. Preintervention, too low CSE (i.e., no MEPs) was noted in participant 2's stronger and weaker hands during RMT assessment. This participant's weaker hand demonstrated some recovery of CSE post-intervention as RMT's MEPs could be elicited at 92% of MSO. Lowered CSE (no MEPs) at 3-month follow-up was noted in participant 8's weaker hand as AMT and RMT could not be recorded.

Relationships between changes in cardiorespiratory fitness (ml min^{-1} $kg^{-1}_{leanmass}$), lean mass (kg), body fat (%), levels of fatigue (FSS, MFIS), workload performed (kcal/session), and TMS changes were investigated with Spearman's coefficient (rho) at the unadjusted significance level of $p < 0.05$. Change scores were calculated as % changes = post – pre/pre.

Differences between TMS values of the stronger and weaker hand were investigated separately for each time point (pre, post, follow-up) with Wilcoxon non-parametric paired *t*-tests.

RESULTS

Exercise Training Increased Corticospinal Excitability in Both Hemispheres

Friedman's test showed a significant difference for AMT between time points (pre, post, follow-up) in both stronger and weaker hands [$\chi^2_{(2)} \geq 8.27, p \leq 0.016$]. Pairwise analysis revealed higher corticospinal excitability (i.e., lower AMT) in participants post- compared to pre-intervention in both stronger [MSO%; Mdn (pre vs. post) = 33 vs. 27, $p = 0.033$] and weaker hands [MSO%; Mdn (pre vs. post) = 41 vs. 37, $p = 0.013$), which returned to baseline at follow-up (**Figures 1A,B**). Higher variability was found for RMT; no change, increases, and decreases of RMT were noted across participants in both hemispheres (stronger and weaker hands), and no statistically significant changes were observed in either hemisphere (**Figures 1C,D**).

Corticospinal gain (excitatory recruitment curve slope) was statistically different between time points in both stronger and weaker hands [$\chi^2_{(2)} \geq 8.40, p \leq 0.015$]. Pairwise analysis revealed increased capacity to recruit excitatory neurons with increased TMS stimulation intensities (i.e., higher slope) post- compared to pre-intervention [Mdn = (pre vs. post) = stronger: 1.33 vs.

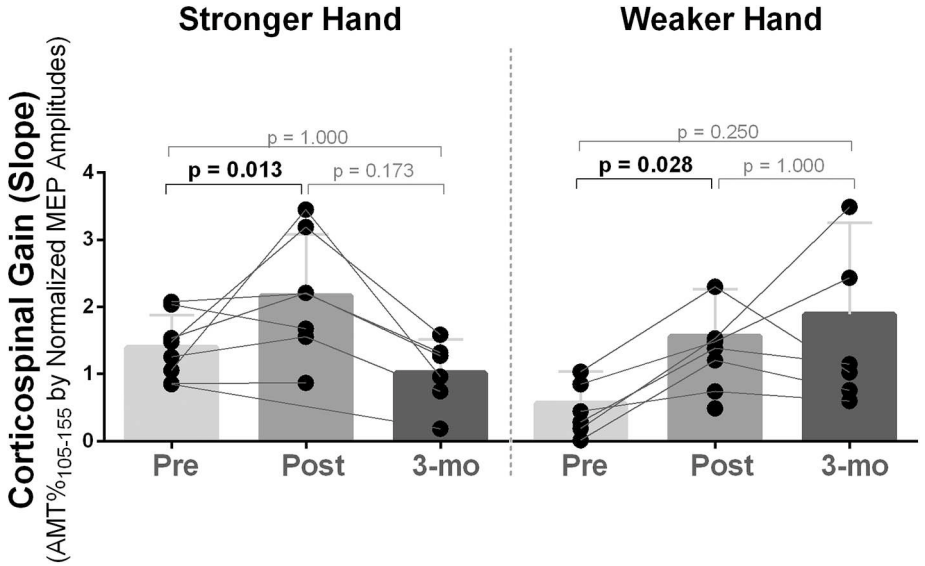


FIGURE 2 | Effects of 10-week treadmill walking exercise training on corticospinal gain. After 10 weeks of exercise training, availability to recruit corticospinal tract neurons with increased transcranial magnetic stimulation intensities was increased (i.e., higher slope) in both brain hemispheres corresponding to the stronger and weaker hands and returned to baseline at 3-month follow-up (3-mo), although, two participants (numbers 6 and 8) continued to increase corticospinal gain in the hemisphere corresponding to the weaker hand during follow-up. The recruitment curve as collected using transcranial magnetic stimulation intensities of 105–155% of the active motor threshold (AMT) (increments of 10%) and the slope was determined from a linear regression between the normalized MEP amplitudes [% of the largest baseline motor-evoked potential (MEP)] against the TMS intensities performed (105–155% of AMT).

2.20, $p = 0.013$; weaker: 0.67 vs. 2.08, $p = 0.028$], which returned to baseline at follow-up (**Figure 2**). Recruitment curve accuracy (R^2) did not change in neither stronger or weaker hand [$\chi^2_{(2)} \leq 4.00$), $p \geq 0.135$].

For MEP amplitudes, statistical significance between time points were noted at the intensities of 135% [$\chi^2_{(2)} = 7.00$, $p = 0.030$] and 145% [$\chi^2_{(2)} = 9.33$, $p = 0.009$] of AMT in the weaker hand and at 145% of AMT in the stronger hand [$\chi^2_{(2)} = 6.00$, $p = 0.050$]. In all cases, pairwise analysis revealed increased corticospinal excitability (higher normalized MEP amplitudes) post- compared to pre-intervention with return to baseline at follow-up [% of largest baseline MEP; Mdn (pre vs. post): weaker hand: 135% of AMT: 85.49 vs. 111.39, $p = 0.028$; 145% of AMT: 85.78 vs. 151.66, $p = 0.012$; stronger hand: 145% of AMT: 88.73 vs. 127.05, $p = 0.048$; **Figure 3**].

Exercise Training Reduced Intracortical Inhibition in the Hemisphere Corresponding to the Stronger Hand

In the stronger hand, differences between time points were noted for CSP investigated in all TMS intensities [105–155% of AMT; $\chi^2_{(2)} \geq 6.00$, $p < 0.050$]. Pairwise analysis revealed reductions in CSP time post- compared to pre-intervention across all intensities used ($p \leq 0.048$), which returned to baseline level at follow-up (**Figure 4A**). In the hemisphere corresponding to the weaker hand, there was a statistically significant difference for CSP time at the different time points at lower TMS intensities {105–125% of AMT [$\chi^2_{(2)} = 6.33$, $p = 0.042$]}; however, statistical significance was not reached during pairwise analysis ($p \geq 0.063$; **Figure 4B**).

Changes in Body Composition, Fitness, and Exercise Performance

Lean body mass of the participants increased from pre- to post-intervention and from post-intervention to follow-up; however, only the change from pre to follow-up was statistically significant [$\chi^2_{(2)} = 7.00$, $p = 0.030$; Mdn, lean mass (kg) (pre vs. follow-up): 41.74 vs. 48.57, $p = 0.028$] (**Figure 5A**). Body fat also decreased during follow-up, and a statistical significance was noted from post to follow-up [$\chi^2_{(2)} = 8.33$, $p = 0.016$; Mdn, body fat % (post vs. follow-up): 40.00 vs. 37.35, $p = 0.012$; **Figure 5B**].

Although four out of eight participants improved their cardiorespiratory fitness (ml min^{-1} $kg^{-1}_{leanmass}$), no overall statistical change was reached ($p \geq 0.368$; **Figure 5D**). However, an increased capacity to perform exercise were noted as participants were able to perform a higher exercise workload (kcal/session) in the last compared to the first exercise session [$\chi^2_{(2)} = 7.14$, $p = 0.028$; Mdn, kcal/session (pre vs. post) = 121.39 vs. 70.24, $p = 0.023$], and this capacity was maintained during follow-up (**Figure 5C**).

Overall Corticospinal Excitation Increased Post-intervention in the Stronger Hand and Was Associated With Reductions in Fatigue

In the stronger hand, overall corticospinal excitation [area under the curve (AUC), normalized MEP amplitudes] differed between

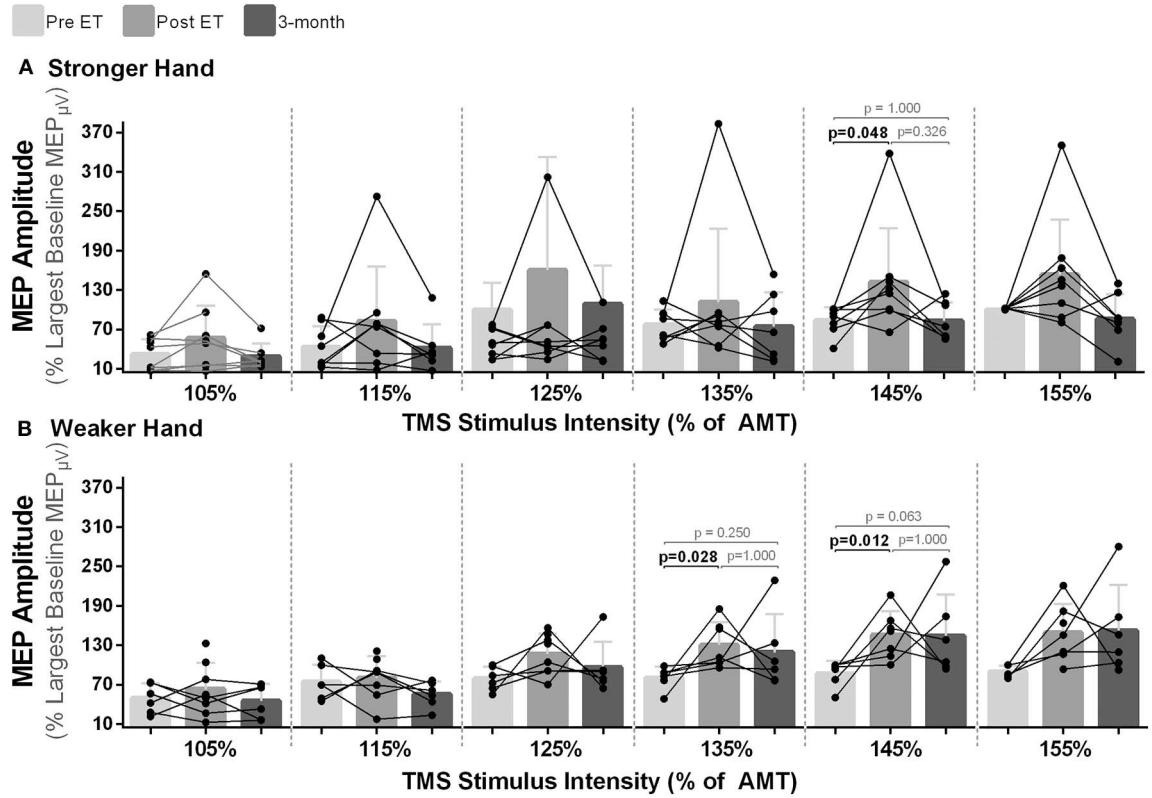


FIGURE 3 | Effects of 10-week treadmill walking exercise training on motor-evoked potential (MEP) amplitudes. **(A)** Higher normalized MEP amplitudes (% of largest baseline MEP) demonstrate higher corticospinal excitability after the exercise training (ET) with return to baseline at 3-month follow-up (3-mo) in the hemisphere corresponding to the stronger hand at a transcranial magnetic stimulation (TMS) intensity of 145% of the active motor threshold (AMT) and **(B)** in the hemisphere corresponding to the weaker hand at the TMS intensities of 135 and 145% of the AMT.

time points [$\chi^2_{(2)} = 11.14$, $p = 0.004$]. Pairwise analysis revealed increased overall corticospinal excitation (higher AUC) post- compared to pre-intervention [Mdn, $AUC_{105-155\%ofAMT}$ (pre vs. post) = 3,237 vs. 3,947, $p \leq 0.016$) with returned to baseline level at follow-up (**Figure 6A**). Relationship analysis demonstrated that greater increases in overall corticospinal excitation in the stronger hand were associated with greater reduction in fatigue severity levels measured with the FSS (rho = 0.762, $p = 0.028$; **Figure 6B**) and fatigue impact measured with the MFIS (rho = 0.962, $p = 0.001$; **Figure 6C**).

Nerve conduction speed (MEP latency) did not change in either side [$\chi^2_{(2)} \leq 1.14$, $p \geq 0.565$; Mdn, milliseconds (pre vs. post vs. follow-up): stronger hand, 24.17 vs. 24.51 vs. 22.12; weaker hand, 26.26 vs. 25.94 vs. 25.97].

All the TMS values (median and range), differences between stronger and weaker hands across time points, and reasons for missing values across time points are reported in **Table 2**.

DISCUSSION

We undertook this study to determine whether a 10-week, 3× /week walking exercise training program would alter corticospinal excitability among people with walking disability due to progressive MS. We report four main findings. First, exercise training resulted in short-term enhancement of corticospinal excitability in both brain hemispheres, which was lost when reassessed during follow-up 3 months later. Second, participants' intracortical inhibition was decreased after training; however, this effect was also short term (lost at follow-up) and was restricted to the hemisphere corresponding to the stronger hand. Third, the training augmented lean mass and reduced body fat, and although there was no change in cardiorespiratory fitness measured as peak of oxygen consumption, capacity to perform exercise (workload) was increased after training and sustained at follow-up (51). Finally, enhancement in corticospinal excitability in the hemisphere corresponding to the stronger hand was correlated with reductions in both severity and impact of fatigue on everyday life (FSS and MFIS, respectively).

Physical Exercise Training to Enhance Corticospinal Excitation in Progressive MS

Motor thresholds and MEP amplitudes are considered indicators of corticospinal excitation, mediated by glutamate and its activity on *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (24, 30). In

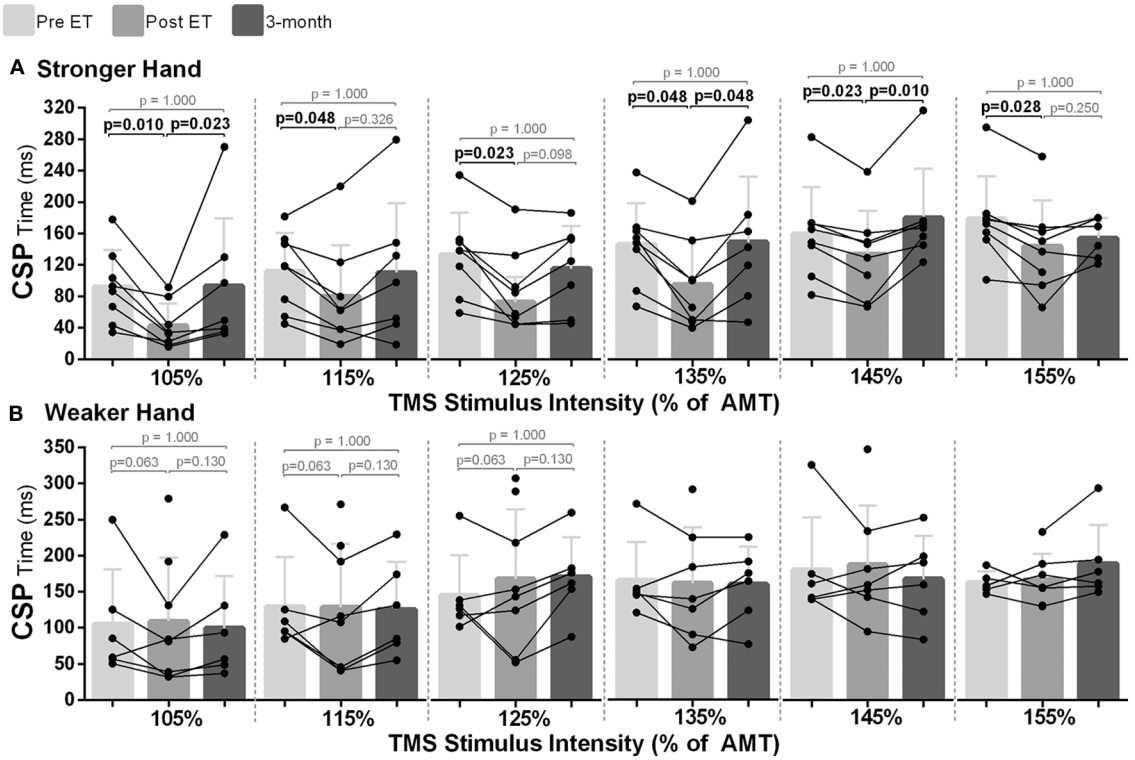


FIGURE 4 | Effects of 10-week treadmill walking exercise training on cortical silent period (CSP) time. **(A)** In the hemisphere corresponding to the stronger hand, shorter CSP time (ms) at all transcranial magnetic stimulation intensities used [105–155% of active motor threshold (AMT)] suggested less GABAergic-mediated intracortical inhibition post-exercise training (ET), with return to baseline at 3-month follow-up (3-mo). **(B)** In the hemisphere corresponding to the weaker hand, although statistical significance was reached for the TMS intensities of 105, 115, and 125% of AMT between the different time points [Friedman's test: pre vs. post vs. 3-mo; $\chi^2_{(2)} = 6.33$, $p = 0.042$], there was no statistical significance during pairwise analysis.

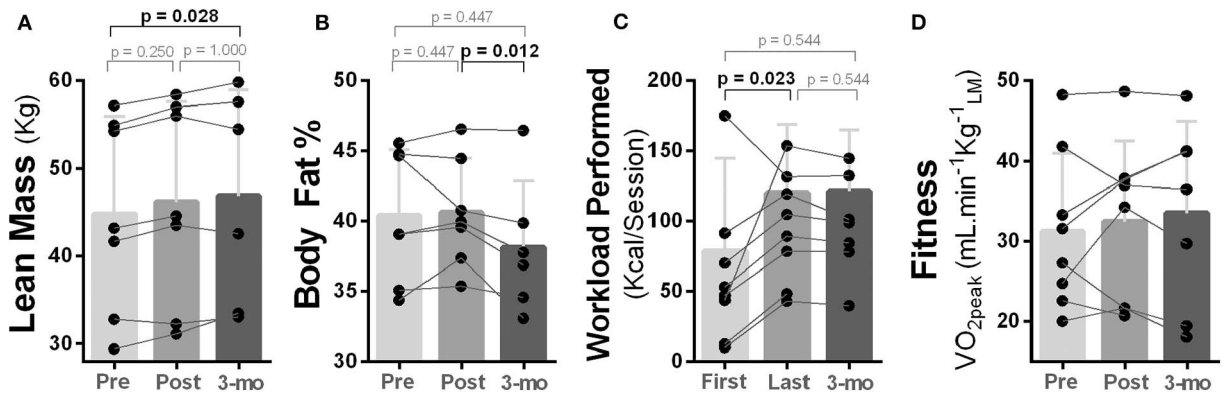


FIGURE 5 | Effects of 10-week treadmill walking exercise training on body composition and physical fitness. **(A)** Amount of lean body mass (kg) measured using dual energy X-ray absorptiometry (DEXA) was higher at 3-month follow-up (3-mo) compared to pre-exercise training. **(B)** Body fat percentage (%) measured using DEXA was lower at 3-month follow-up compared to post-exercise training. **(C)** Participants were able to perform a higher exercise workload (kcal/session) at their last exercise session compared to the first. Total amount of workload performed was estimated using standardized equations (49). **(D)** No change was noted for cardiorespiratory fitness measured as peak rate of oxygen uptake during a graded maximal exercise test [VO_{2peak} = ml min^{-1} $kg^{-1}_{ofleanmass(LM)}$].

fact, higher glutamatergic receptor activity is associated with greater capacity for synaptic plasticity (72, 73), and disruption of this excitatory circuitry is responsible for diminished neuroplasticity and lower capacity to learn new tasks and recover from neurological damage (e.g., aging, stroke, MS) (4, 7, 71). Therefore, there are important initiatives underway to develop

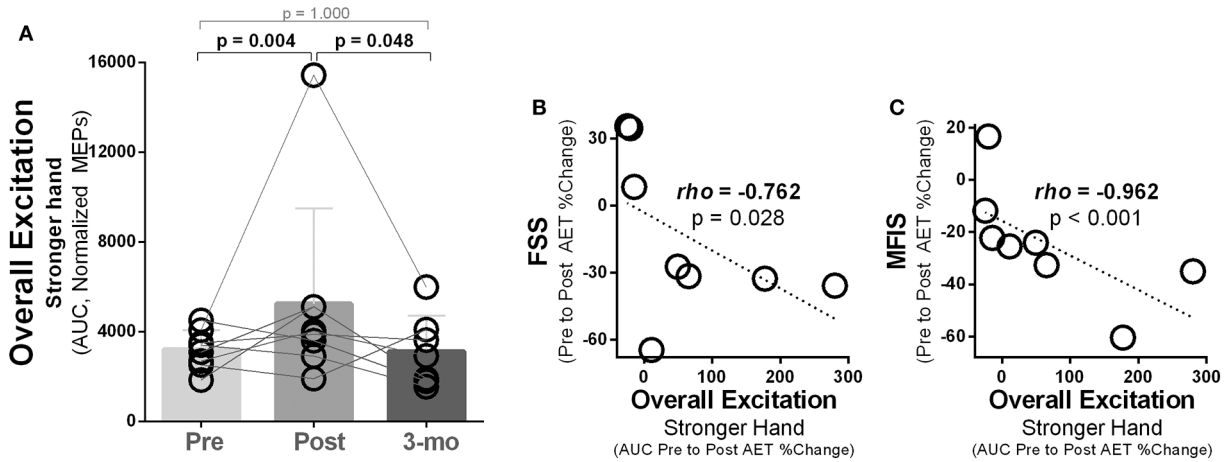


FIGURE 6 | Ten weeks of treadmill walking exercise training induced increased overall corticospinal excitation that was associated with reductions in subjective fatigue. **(A)** In the hemisphere corresponding to the stronger hand, higher overall corticospinal excitation was noted post-exercise training, with complete return to baseline during 3-month follow-up (3-mo). Overall excitation was calculated as the area under the curve (AUC) using the trapezoid rule $\Delta X \times (Y1 + Y2)/2$, with X being the transcranial magnetic intensities used (105–155% of AMT; increments of 10%) and Y being the normalized motor-evoked potential (MEP) amplitudes (% of largest baseline MEP). **(B)** Increases in overall excitation (AUC) in the hemisphere corresponding to the stronger hand were associated to reductions in subjective levels of fatigue measured using the fatigue severity scale (FSS) and **(C)** the modified impact scale (MFIS).

new treatments (e.g., exercise, pharmacological, non-invasive brain stimulation) aimed at increasing glutamatergic-mediated brain excitation in the injured brain to enhance neuroplasticity and recover function (9, 45, 71, 74–76). For instance, studies using TMS have confirmed that, in comparison to those who are less physically active, individuals with higher fitness have lower motor thresholds and higher MEP amplitudes (29) (i.e., higher corticospinal excitability) and demonstrate superior increases in MEP amplitudes (i.e., greater neuroplastic response) following paired associative stimulation to induce neuroplasticity (28, 77).

We have previously shown that acute exercise increases corticospinal excitation (i.e., higher MEP amplitude) and reduces intracortical inhibition (i.e., shorter CSP) among people with walking disability due to progressive MS (47). Importantly, this effect was noted only in the stronger hand (47), likely due to a more intact (i.e., less affected) contralateral corticospinal representation (33). Here, we showed bilateral reductions in AMT, increases in MEP amplitudes, and superior motor neuronal recruitment (higher recruitment curve slope) after 10 weeks of aerobic exercise training. This suggests that the stimulus from regular exercise training may have led to the chronic enhancements in excitatory synaptic transmission noted in these participants. Moreover, even though the hemisphere corresponding to the weaker hand, which was likely more affected by MS (33, 78), was unresponsive after one exercise session (47), in this longer term exercise training, it demonstrated capacity to improve in synaptic excitatory transmission. It is interesting to observe that Nicoletti et al. (9) recently reported enhanced corticospinal excitation in people with progressive MS after 4 weeks of D-aspartate treatment, which aimed to enhance NMDA receptor activity (9). They also showed increases in MEP amplitudes following intermittent theta burst stimulation (i.e., enhanced neuroplasticity) (9). It appears that exercise training has comparable benefits in terms of enhancing capacity for neuroplasticity in progressive MS. It is important to note that the corticospinal excitability enhancements reported here and those by Nicoletti et al. (9) were short term and disappeared 3 months after cessation of the intervention. Therefore, we suggest that treatments that enhance neuroplasticity, such as physical exercise training, should be prescribed continuously in progressive MS to protect the brain, improve brain function, and likely to potentiate the effects of treatments (e.g., drugs) and other neuroplasticity-inducing protocols (e.g., non-invasive brain stimulation).

Physical Exercise Training to Reduce Intracortical Inhibition in Progressive MS

When applying suprathreshold TMS stimulations to the primary motor cortex with participants performing a tonic muscle contraction of the contralateral target muscle, the length of the period of cessation of muscle activity (CSP) is an indicator of intracortical inhibition mediated by the activity of the inhibitory neurotransmitter GABA on its ionotropic and metabotropic receptors ($GABA_A$ and $GABA_B$, respectively) (24, 32). Although the cortical and spinal contribution to the CSP length is still unclear (24, 79), it is generally accepted that the cortex is the main modulator of CSP change (32). Because excessive GABAergic-mediated intracortical inhibition is considered pathological (80, 81), detrimental to neuroplasticity (39, 40, 81, 82), and is associated with disease progression in MS (36) and stroke (83), decreasing its activity is an attractive treatment strategy to boost neuroplasticity (40, 81).

In healthy people and people with stroke, studies have confirmed that even a single bout of aerobic exercise is able to acutely reduce short intracortical inhibition (59, 83–86) assessed with TMS paired pulse, a TMS biomarker of

TABLE 2 | Transcranial magnetic stimulation values between stronger and weaker sides.

Median (range)	Pre training			Post training			3-month follow up		
TMS variable	**Stronger**	**Weaker**	**Sig.**	**Stronger**	**Weaker**	**Sig.**	**Stronger**	**Weaker**	**Sig.**
RMT (MSO%)	40 (28–68)[a]	45 (30–73)[a]	0.618	37 (22–76)	48 (26–92)	0.205	43 (24–56)[d]	40 (29–81)[f]	0.138
AMT (MSO%)	33 (20–64)	42 (27–100)	0.058	27 (17–45)	37 (18.76)	0.042*	30 (20–60)[e]	31 (21–64)[f]	0.307
$MEP_{105\%AMT}$	231.13 (186.67–331.17)	415.5 (181.5–464.25)[b]	0.046*	477.18 (243.50–1097.17)	222.50 (124.17–1072.20)	0.012	374.60 (91.50–634.40)[e]	295.88 (165.33–358.67)[f]	0.116
$MEP_{115\%AMT}$	310.00 (96.75–1398.00)	593.05 (174.00–1130.00)[b]	0.463	621.21 (319.00–1422.75)	320.75 (172.75–1720.80)	0.050	430.75 (146.25–1360.50)[e]	370.42 (153.00–860.33)[f]	0.600
$MEP_{125\%AMT}$	344.92 (199.50–2640.00)	818.47 (161.00–1365.77)[b]	0.753	740.50 (209.47–1592.00)	510–13 (226.60–3030.33)	0.779	597.40 (213.20–2100.00)[e]	772.17 (228.67–1453.75)[f]	0.753
$MEP_{135\%AMT}$	672.58 (248.00–3546.00)	550.23 (206.00–1483.67)[b]	0.345	1348.75 (353.33–1722.25)	665.50 (237.75–3587.33)[c]	0.237	724.20 (117.00–4664.40)[e]	994.67 (232.67–2159.67)[f]	0.463
$MEP_{145\%AMT}$	568.00 (334.50–3727.80)	564.63 (248.00–1812.67)[b]	0.345	1784.88 (430.33–4608.00)	765.67 (310.50–3998.00)[c]	0.128	1065.67 (272.00–4634.00)[e]	1165.08 (260.33–2814.80)[f]	0.345
$MEP_{155\%AMT}$	1037.55 (357.00–3771.33)	870.67 (468.50–1933.00)[b]	0.686	2047.85 (373.75–4031.20)	892.50 (232.33–4268.00)[c]	0.091	1346.17 (98.00–4669.00)[e]	1252.75 (257.20–2798.00)[f]	0.249
eREC slope (gain)	14.80 (3.53–77.38)	3.14 (−1.83–30.00)[b]	0.075	28.41 (3.22–82.06)	10.18 (1.70–66.77)[c]	0.091	15.51 (0.90–49.28)[e]	19.11 (2.24–59.03)[f]	0.686
eREC R^2 (accuracy)	0.77 (0.51–0.97)	0.35 (0.00–0.97)[b]	0.173	0.76 (.042–0.96)	0.82 (0.66–0.99)[c]	0.499	0.78 (0.05–0.87)[e]	0.91 (0.82–0.95)[f]	0.043*
eREC AUC (overall excitation)	25852 (13182–126385)	30498 (7558–65129)[b]	0.463	58744.17 (16940.42–108246.00)	30189.83 (11258.50–150065.17)[c]	0.176	34050.50 (8432.00–154112.00)[e]	40965.42 (10859.33–83448.00)[f]	0.463
$CSP_{105\%AMT}$	89.56 (34.65–177.40)	72.47 (50.61–249.76)[b]	0.249	33.35 (16.17–91.63)	82.77 (31.92–279.04)	0.012*	49.66 (32.80–269.32)[e]	75.26 (37.17–229.93)[f]	0.249
$CSP_{115\%AMT}$	118.31 (45.06–181.49)	102.27 (84.86–266.75)[b]	0.345	62.34 (19.35–219.98)	112.16 (40.96–271.27)	0.036*	98.03 (18.88–279.07)[e]	108.28 (55.22–229.40)[f]	0.046*
$CSP_{125\%AMT}$	138.49 (58.90–233.98)	128.85 (101.84–255.44)[b]	0.116	71.41 (44.46–190.77)	148.58 (52.44–307.23)	0.017*	124.91 (45.60–186.06)[e]	169.13 (87.86–259.93)[f]	0.046*
$CSP_{135\%AMT}$	151.60 (67.87–237.88)	150.01 (121.46–272.03)[b]	0.345	83.57 (40.51–201.44)	140.24 (73.29–292.23)[c]	0.018*	142.76 (47.85–304.16)[e]	170.69 (77.56–225.96)[f]	0.173
$CSP_{145\%AMT}$	157.33 (82.26–282.32)	151.81 (139.38–325.93)[b]	0.173	137.96 (67.22–238.43)	159.61 (94.94–347.51)[c]	0.018*	167.32 (123.66–316.11)[e]	175.15 (83.65–252.69)[f]	0.345
$CSP_{155\%AMT}$	174.75 (101.28–294.92)	158.33 (146.92–187.09)[b]	0.893	143.82 (66.13–257.88)	156.08 (129.73–233.08)[c]	0.028*	156.71 (121.33–179.94)[e]	169.94 (149.51–293.69)[f]	0.046*
iREC slope (Gain)	1.88 (0.91–3.68)	1.84 (0.83–2.19)[b]	0.893	2.04 (0.89–2.56)	1.97 (0.29–2.88)[c]	0.866	2.17 (0.75–2.73)[e]	1.76 (1.03–2.27)[f]	0.345
iREC R^2 (accuracy)	0.94 (0.75–0.99)	0.88 (0.84–0.99)[b]	0.893	0.88 (0.67–0.97)	0.87 (0.01–0.95)[c]	0.237	0.90 (0.73–0.93)[e]	0.70 (0.54–0.79)[f]	0.028*
iREC AUC (overall Inhibition)	6975.5 (3262.0–11718.0)	6531.5 (5823.00–12450.30)[b]	0.249	4369.13 (2271.20–10254.75)	6666.25 (3601.05–13043.85)[c]	0.018*	5857.20 (3397.85–7825.15)[e]	7482.20 (3976.30–12292.90)[f]	0.116
MEP latency (ms)	24.17 (21.38–43.15)[a]	26.26 (20.45–35.52)[a]	0.866	24.51 (19.48–43.78)	25.95 (20.36–38.02)	1.000	22.12 (21.88–29.69)[e]	25.97 (20.26–28.20)[f]	0.686

AMT, active motor threshold; CSP, cortical silent period; eREC, excitatory recruitment curve; iREC, inhibitory recruitment curve; MEP, motor evoked potential; MSO%, maximal stimulator output percentage; RMT, resting motor threshold; eREC Slope = MEP Amplitude (μV) by TMS intensity$_{105-155\%AMT}$; iREC Slope = CSP time (ms) by TMS intensity$_{105-155\%AMT}$; Area under the curve (AUC) was calculated for both excitatory and inhibitory RECs using the trapezoid rule ΔX x (Y1+Y2)/2, whereby X were the MSO% used (i.e., X axis values, 105–155% of AMT) and Y are the recorded CSP lengths (ms) or the MEP amplitudes (μV).

**Difference between stronger and weaker hand is statistically significant at $\alpha < 0.05$.*

[a] Missing data from participant 2 due to too low corticospinal excitability (i.e., no resting MEPs).

[b] Missing data from participant 2 and 7 due to too high AMT (AMT = 100 and 82%, respectively), thus the required increases in MSO% based on AMT to assess the REC could not be performed).

[c] Missing data from participant 7 due to high AMT (AMT = 76%), thus the required intensities of 135–155% of AMT could not be performed, and the slope, R^2 and AUC could not be calculated).

[d] Time point with n = 5 (participant 2 could not be reached during follow-up assessment, missing data from participant 7 and 6 due to too low corticospinal excitability (i.e., no resting MEPs) and overheating of equipment (i.e., stimulator).

[e] Missing data from participant 2 (could not be reached during follow-up).

[f] Missing data from participant 2 (could not be reached during follow-up) and 7 [too low corticospinal excitability (i.e., no resting or contracting MEPs (RMT and AMT)].

$GABA_A$-receptor activity (24). We recently reported a similar effect after acute aerobic exercise in people with progressive MS (47). Interestingly, here, we showed that after 10 weeks of exercise training, CSP duration was reduced at all TMS intensities, indicating reductions in both $GABA_A$ and $GABA_B$-mediated intracortical inhibition. This result aligns with findings in healthy individuals demonstrating that 4–12 weeks of strength exercise training reduced both $GABA_A$- and $GABA_B$-receptor activity, as decreasing in short-intracortical inhibition and duration of the CSP elicited at higher TMS intensities, respectively (26). We have previously shown that among people with MS, superior cardiorespiratory fitness was related to shorter CSP (44). In our present findings, although there were no significant improvements in cardiorespiratory fitness measured as the peak of oxygen consumption (VO_{2peak}), there were other indicators of improved physical health (48) such as higher capacity to perform exercise (i.e., kcal/session), greater lean mass, and lower body fat percentage, and increases in other parameters of cardiorespiratory fitness such as the oxygen uptake efficiency slope [for details, see (51)]. The fact that the beneficial reduction (acute and long term) in intracortical inhibition was only observed in the brain hemisphere corresponding to the stronger hand may suggest a greater neuroplastic potential of inhibitory mechanisms in the hemisphere thought to be less affected by MS. Furthermore, our walking training provided a high degree of task-specific training (18, 87, 88). Ziemann et al. has shown that less GABAergic-mediated intracortical inhibition, assessed with TMS, was essential for motor learning processes from task-specific training to occur (89). Decreasing GABAergic-mediated intracortical inhibition has also been proposed to be an important factor initiating increases in muscular strength (26, 27, 31). Although we did not measure muscular strength (e.g., MVC pre–posttraining), we did note increases in lean mass at post and follow-up as well as improvements in walking function [e.g., walking speed; see (51)]. Altogether, this indicates that long-term physical exercise that utilizes task-specific training in highly disabled people with progressive MS reduces intracortical inhibition and possibly improves and restores physical function through enhanced neuroplasticity. Although, because no correlation between changes in intracortical inhibition, body composition, and walking function was noted, it remains to be answered whether decreasing intracortical inhibition would lead to improvements in learning and restoration of function in people with MS. Future research should examine whether such effects would take place in a larger sample with different walking abilities using a randomized controlled design. As well, because we measured overall gains in walking function (51) and body composition, future research should examine whether the enhanced plasticity (reduced inhibition) measured in the hemisphere corresponding to the stronger side of the body indeed translates into global brain function improvement (60) (e.g., bilateral and cognitive function) or whether it is restricted to the contralateral representation. This would be an important discovery for interventions aiming at improving function of the most affected side.

It is interesting that, when compared to healthy controls, some studies have shown reduced intracortical inhibition (shorter CSP) in MS patients (90, 91). Nantes et al. reported that shorter CSP correlated with lower whole brain cortical volume (MRI, magnetic transfer ratio) in progressive MS and that, interestingly, longer CSP was a predictor of upper extremity motor dysfunction (92). Therefore, when compared to the healthy central nervous system (CNS), the CNS affected by MS may display decreased activity of inhibitory mechanisms that, curiously, may work as a compensatory mechanism during brain disease. The concept that there are compensatory mechanisms that increase brain excitation and decrease brain inhibition in order to preserve brain function in CNS disease has been recently proposed by other authors (7, 33, 44, 93–95). However, these processes are certainly not uniform across CNS disorders. For instance, in Parkinson's disease, Fisher et al. (96) showed that high-intensity treadmill exercise program improved walking performance and lengthened CSP time (96), which is typically shortened in people with Parkinson's disease (97). Thomas et al. (98) also showed lengthening of CSP in people with incomplete spinal cord injury after a regimen of treadmill training. Although the mechanisms are not entirely clear, our work and the work of others suggests that rehabilitation and exercise prime the CNS as measured by shifting of the CSP.

Corticospinal Excitability and Fatigue in MS

Fatigue is one of the most disabling symptoms in MS (44–46). Although the etiology of MS-related fatigue is not completely understood, neuroimaging studies [e.g., MRI, functional MRI (fMRI)] have proposed that its development and progression is due to structural and functional abnormalities in both cortical and subcortical areas (45). Previous studies have shown that 10–12 weeks of physical exercise training can lessen subjective fatigue in people with MS (99), including progressive MS (51, 100). Based on previous findings showing an association between shorter CSP and lowered levels of subjective fatigue in a cohort of people with MS (44), we proposed that improving fitness through exercise training could mitigate fatigue by decreasing GABAergic-mediated intracortical inhibition (i.e., shortening CSP). In this current pilot study, we reported a strong association between increases in corticospinal excitation (recruitment curve; AUC) and reductions in subjective fatigue (FSS and MFIS). Nicoletti et al. (9) also demonstrated reductions in subjective fatigue (FSS) and increases in corticospinal excitation (intracortical facilitation) after D-aspartate treatment in people with progressive MS (9). Furthermore, Créange et al. (101) have also shown increases in corticospinal excitation (e.g., RMT reduction) and reduction in levels of fatigue after erythropoietin treatment to improve walking in people with progressive MS. Our results and the results of others support that there is a link between corticospinal excitation/inhibition and fatigue, which should be examined in larger trials. In fact, non-invasive brain stimulation methods (repetitive TMS, transcranial direct current stimulation), which aim to increase

cortical excitation and treat MS fatigue, have been recently proposed (45). It is important to note that the abovementioned experiments, and the present study, measured perceived (i.e., subjective) fatigue and not fatigability (i.e., muscle/performance fatigability measured during contraction). Nonetheless, because perceived fatigue and fatigability closely associate (102), our results showing reduced levels of perceived fatigue and improved fitness suggests that following training, subjects required less physical effort to perform activities of daily living, suggesting superior energy availability and reduced fatigability (102). Therefore, we propose that exercise training might be able to mitigate symptoms of fatigue possibly by acting through increases in excitatory circuitry.

Limitations

There are some important limitations to consider when interpreting the results of the present study. First, this was a small pilot study, and no statistical sample size calculation was conducted for the outcomes presented in this manuscript, which limits the statistical power to obtain conclusive results. Second, no control group was included, which limits the conclusion on the true effect of the intervention. Third, as only patients with progressive MS and severe MS-related walking disabilities (EDSS, 6.0–6.5) were included, the findings may not be applicable for relapsing–remitting and/or less disabled MS patients. Despite these limitations, the novel insights from this study may serve as a rationale for larger studies and continued efforts in investigating the effects of exercise and physical rehabilitation on neuroplasticity and functional recovery in MS.

As for considerations for future studies, although the aim of this study was to investigate changes in corticospinal excitability in a non-exercised hand muscle to demonstrate widespread effects of exercise training on global brain plasticity (59, 60), investigating muscles that were more involved in the walking training (e.g., lower limb muscles) could provide more insight regarding the link between the trained muscle and cortical function (TMS) (27). Moreover, having participants' neuroimaging data (e.g., magnetic resonance imaging) could help to better understand the role of lesion volume and location on exercise-induced corticospinal excitability changes. We determined averaged MEP amplitudes and CSP times from a small number of trials (three to six) as done previously by others (28, 65, 66), and with participants performing tonic contraction, in order to reduce intrasubject variability (27). Future studies should examine the optimal number of stimulation trials (103) in order to produce reliable MEP/CSP data. With respect to the TMS recruitment curve parameters, we used linear regression (TMS intensities by MEP amplitudes), as done by others (25, 34), in an attempt to assess the corticospinal tract recruitment gain (slope) and accuracy (R^2); biomarkers were previously proposed by Potter-Baker et al. (67) to reflect morpho-physiological integrity of the corticospinal tract in stroke. However, more studies are necessary in order to understand what the best model is [e.g., sigmoidal (67) or linear (25, 34)] when calculating these parameters while taking into consideration the different TMS methodologies (e.g., range of TMS intensities employed), the clinical population (e.g., stroke, MS), and lesion profile (e.g., lesion volume, location).

CONCLUSION

To our knowledge, this is the first study to investigate longer term effects of exercise on corticospinal function using TMS in patients with progressive MS. This exploratory pilot study provides evidence that a neuroplastic potential still exists in patients with progressive MS and severe MS-related walking disability. Specifically, we found that 10 weeks of vigorous treadmill training reduced intracortical inhibition and increased corticospinal excitability. These corticospinal adaptations were predominately found in the brain hemisphere corresponding to the stronger hand, suggesting a greater neuroplastic potential in the hemisphere that may be less affected by MS. Moreover, the exercise-induced enhancement in cortical excitation was associated with reductions in fatigue, suggesting this as a potential mechanism involved in the effects of exercise on fatigue. The novel findings from this pilot study highlight the importance of long-term exercise efforts—even in patients with progressive MS—and can serve as a rationale for future studies and continued efforts in investigating the effects of exercise on the brain.

DATA AVAILABILITY STATEMENT

The data supporting this study are available at request from the corresponding author at the Memorial University of Newfoundland, Canada.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Health Research Ethics Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AC, AD, and MP: conception or design of the research. AC, RP, and AD: data collection. AC and AD: data cleaning and analysis. AC, MR, and MP: writing and editing the manuscript. All authors interpretation of data, final approval and revision of the version to be published, and agreement to be accountable for all aspects of the work.

FUNDING

This study was funded by the Program of Experimental Medicine Graduate Scholarship (AD), O'Dea Research Fellowship (AD, AC), Translational and Personalized Medicine Initiative (AD), Canada Research Chair (MP; 230457), and Canada Foundation for Innovation (MP: 33621).

REFERENCES

1. Thompson AJ, Baranzini SE, Geurts J, Hemmer B, Ciccarelli O. Multiple sclerosis. *Lancet.* (2018) 391:1622–36. doi: 10.1016/S0140-6736(18)30481-1
2. Dutta R, Trapp BD. Relapsing and progressive forms of multiple sclerosis: insights from pathology. *Curr Opin Neurol.* (2014) 27:271–8. doi: 10.1097/WCO.0000000000000094
3. Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. *Lancet Neurol.* (2015) 14:183–93. doi: 10.1016/S1474-4422(14)70256-X
4. Weiss S, Mori F, Rossi S, Centonze D. Disability in multiple sclerosis: when synaptic long-term potentiation fails. *Neurosci Biobehav Rev.* (2014) 43:88–99. doi: 10.1016/j.neubiorev.2014.03.023
5. Ciotti JR, Cross AH. Disease-modifying treatment in progressive multiple sclerosis. *Curr Treat Options Neurol.* (2018) 20:12. doi: 10.1007/s11940-018-0496-3
6. Ayache SS, Chalah MA. Cortical excitability changes: a mirror to the natural history of multiple sclerosis? *Neurophysiol Clin.* (2017) 47:221–3. doi: 10.1016/j.neucli.2017.02.001
7. Stampanoni Bassi M, Leocani L, Comi G, Iezzi E, Centonze D. Can pharmacological manipulation of LTP favor the effects of motor rehabilitation in multiple sclerosis? *Mult Scler.* (2018) 24:902–7. doi: 10.1177/1352458517721358
8. Thompson AJ. Challenge of progressive multiple sclerosis therapy. *Curr Opin Neurol.* (2017) 30:237–40. doi: 10.1097/WCO.0000000000000453
9. Nicoletti CG, Monteleone F, Marfia GA, Usiello A, Buttari F, Centonze D, et al. Oral D-Aspartate enhances synaptic plasticity reserve in progressive multiple sclerosis. *Mult Scler J.* (2019) 26:304–11. doi: 10.1177/1352458519828294
10. Ayache SS, Créange A, Farhat WH, Zouari HG, Lesage C, Palm U, et al. Cortical excitability changes over time in progressive multiple sclerosis. *Funct Neurol.* (2015) 30:257–63. doi: 10.11138/FNeur/2015.30.4.257
11. Voss MW, Vivar C, Kramer AF, van Praag H. Bridging animal and human models of exercise-induced brain plasticity. *Trends Cogn Sci.* (2013) 17:525–44. doi: 10.1016/j.tics.2013.08.001
12. Ploughman M, Austin MW, Glynn L, Corbett D. The effects of poststroke aerobic exercise on neuroplasticity: a systematic review of animal and clinical studies. *Transl Stroke Res.* (2015) 6:13–28. doi: 10.1007/s12975-014-0357-7
13. Lin T-W, Tsai S-F, Kuo Y-M. Physical exercise enhances neuroplasticity and delays Alzheimer's disease. *Brain Plast.* (2018) 4:95–110. doi: 10.3233/BPL-180073
14. Ploughman M, Eskes GA, Kelly LP, Kirkland MC, Devasahayam AJ, Wallack EM, et al. Synergistic benefits of combined aerobic and cognitive training on fluid intelligence and the role of IGF-1 in chronic stroke. *Neurorehabil Neural Repair.* (2019) 33:199–212. doi: 10.1177/1545968319832605
15. Filli L, Schwab ME. Structural and functional reorganization of propriospinal connections promotes functional recovery after spinal cord injury. *Neural Regen Res.* (2015) 10:509–13. doi: 10.4103/1673-5374.155425
16. Langeskov-Christensen M, Heine M, Kwakkel G, Dalgas U. Aerobic capacity in persons with multiple sclerosis: a systematic review and meta-analysis. *Sports Med.* (2015) 45:905–23. doi: 10.1007/s40279-015-0307-x
17. Riemenschneider M, Hvid LG, Stenager E, Dalgas U. Is there an overlooked "window of opportunity" in MS exercise therapy? Perspectives for early MS rehabilitation. *Mult Scler.* (2018) 24:886–94. doi: 10.1177/1352458518777377
18. Devasahayam AJ, Downer MB, Ploughman M. The effects of aerobic exercise on the recovery of walking ability and neuroplasticity in people with multiple sclerosis: a systematic review of animal and clinical studies. *Mult Scler Int.* (2017) 2017:4815958. doi: 10.1155/2017/4815958
19. Heine M, van de Port I, Rietberg MB, van Wegen EE, Kwakkel G. Exercise therapy for fatigue in multiple sclerosis. *Cochrane Database Syst Rev.* (2015) 2015:CD009956. doi: 10.1002/14651858.CD009956.pub2
20. Motl RW, Pilutti LA. The benefits of exercise training in multiple sclerosis. *Nat Rev Neurol.* (2012) 8:487–97. doi: 10.1038/nrneurol.2012.136
21. Mark VW, Taub E, Uswatte G, Morris DM, Cutter GR, Adams TL, et al. Phase II randomized controlled trial of constraint-induced movement therapy in multiple sclerosis. Part 1: effects on real-world function. *Neurorehabil Neural Repair.* (2018) 32:223–32. doi: 10.1177/1545968318761050
22. Rasova K, Prochazkova M, Tintera J, Ibrahim I, Zimova D, Stetkarova I. Motor programme activating therapy influences adaptive brain functions in multiple sclerosis: clinical and MRI study. *Int J Rehabil Res.* (2015) 38:49–54. doi: 10.1097/MRR.0000000000000090
23. Bonzano L, Pedullà L, Tacchino A, Brichetto G, Battaglia MA, Mancardi GL, et al. Upper limb motor training based on task-oriented exercises induces functional brain reorganization in patients with multiple sclerosis. *Neuroscience.* (2019) 410:150–9. doi: 10.1016/j.neuroscience.2019.05.004
24. Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin Neurophysiol.* (2015) 126:1071–107. doi: 10.1016/j.clinph.2015.02.001
25. Schättin A, Gennaro F, Egloff M, Vogt S, de Bruin ED. Physical activity, nutrition, cognition, neurophysiology, and short-time synaptic plasticity in healthy older adults: a cross-sectional study. *Front Aging Neurosci.* (2018) 10:242. doi: 10.3389/fnagi.2018.00242
26. Kidgell DJ, Bonanno DR, Frazer AK, Howatson G, Pearce AJ. Corticospinal responses following strength training: a systematic review and meta-analysis. *Eur J Neurosci.* (2017) 46:2648–61. doi: 10.1111/ejn.13710
27. Kidgell DJ, Pearce AJ. What has transcranial magnetic stimulation taught us about neural adaptations to strength training? A brief review. *J Strength Cond Res.* (2011) 25:3208–17. doi: 10.1519/JSC.0b013e318212de69
28. Lulic T, El-Sayes J, Fassett HJ, Nelson AJ. Physical activity levels determine exercise-induced changes in brain excitability. *PLoS ONE.* (2017) 12:e0173672. doi: 10.1371/journal.pone.0173672
29. Monda V, Valenzano A, Moscatelli F, Salerno M, Sessa F, Triggiani AI, et al. Primary motor cortex excitability in karate athletes: a transcranial magnetic stimulation study. *Front Physiol.* (2017) 8:695. doi: 10.3389/fphys.2017.00695
30. Stagg CJ, Bestmann S, Constantinescu AO, Moreno LM, Allman C, Mekle R, et al. Relationship between physiological measures of excitability and levels of glutamate and GABA in the human motor cortex. *J Physiol.* (2011) 589:5845–55. doi: 10.1113/jphysiol.2011.216978
31. Kidgell DJ, Pearce AJ. Corticospinal properties following short-term strength training of an intrinsic hand muscle. *Hum Mov Sci.* (2010) 29:631–41. doi: 10.1016/j.humov.2010.01.004
32. Ziemann U, Wolters A, Ziemann U, Benecke R. The cortical silent period. In: Epstein CM, Wassermann EM, Ziemann U, editors. *Oxford Handbook of Transcranial Stimulation*. New York, NY: Oxford University Press (2012). doi: 10.1093/oxfordhb/9780198568926.013.0010
33. Chaves AR, Wallack EM, Kelly LP, Pretty RW, Wiseman HD, Chen A, et al. Asymmetry of brain excitability: a new biomarker that predicts objective and subjective symptoms in multiple sclerosis. *Behav Brain Res.* (2019) 359:281–91. doi: 10.1016/j.bbr.2018.11.005
34. Neva JL, Lakhani B, Brown KE, Wadden KP, Mang CS, Ledwell NH, et al. Multiple measures of corticospinal excitability are associated with clinical features of multiple sclerosis. *Behav Brain Res.* (2016) 297:187–95. doi: 10.1016/j.bbr.2015.10.015
35. Nantes JC, Zhong J, Holmes SA, Whatley B, Narayanan S, Lapierre Y, et al. Intracortical inhibition abnormality during the remission phase of multiple sclerosis is related to upper limb dexterity and lesions. *Clin Neurophysiol.* (2016) 127:1503–11. doi: 10.1016/j.clinph.2015.08.011
36. Tataroglu C, Genc A, Idiman E, Cakmur R, Idiman F. Cortical silent period and motor evoked potentials in patients with multiple sclerosis. *Clin Neurol Neurosurg.* (2003) 105:105–10. doi: 10.1016/S0303-8467(02)00127-0
37. Classen J, Schnitzler A, Binkofski F, Werhahn KJ, Kim YS, Kessler KR, et al. The motor syndrome associated with exaggerated inhibition within the primary motor cortex of patients with hemiparetic. *Brain.* (1997) 120(Pt 4):605–19. doi: 10.1093/brain/120.4.605
38. Gray WA, Palmer JA, Wolf SL, Borich MR. Abnormal EEG responses to TMS during the cortical silent period are associated with hand function in chronic stroke. *Neurorehabil Neural Repair.* (2017) 31:666–76. doi: 10.1177/1545968317712470
39. Sale MV, Ridding MC, Nordstrom MA. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by

paired associative stimulation. *Exp Brain Res.* (2007) 181:615–26. doi: 10.1007/s00221-007-0960-x
40. Sale A, Berardi N, Maffei L. Environment and brain plasticity: towards an endogenous pharmacotherapy. *Physiol Rev.* (2014) 94:189–234. doi: 10.1152/physrev.00036.2012
41. Snow NJ, Wadden KP, Chaves AR, Ploughman M. Transcranial magnetic stimulation as a potential biomarker in multiple sclerosis: a systematic review with recommendations for future research. *Neural plast.* (2019) 2019:6430596. doi: 10.1155/2019/6430596
42. Beaulieu LD, Milot MH. Changes in transcranial magnetic stimulation outcome measures in response to upper-limb physical training in stroke: a systematic review of randomized controlled trials. *Ann Phys Rehabil Med.* (2018) 61:224–34. doi: 10.1016/j.rehab.2017.04.003
43. Abraha B, Chaves AR, Kelly LP, Wallack EM, Wadden KP, McCarthy J, et al. A bout of high intensity interval training lengthened nerve conduction latency to the non-exercised affected limb in chronic stroke. *Front Physiol.* (2018) 9:827. doi: 10.3389/fphys.2018.00827
44. Chaves AR, Kelly LP, Moore CS, Stefanelli M, Ploughman M. Prolonged cortical silent period is related to poor fitness and fatigue, but not tumor necrosis factor, in multiple sclerosis. *Clin Neurophysiol.* (2019) 130:474–83. doi: 10.1016/j.clinph.2018.12.015
45. Chalah MA, Riachi N, Ahdab R, Créange A, Lefaucheur JP, Ayache SS. Fatigue in multiple sclerosis: neural correlates and the role of non-invasive brain stimulation. *Front Cell Neurosci.* (2015) 9:460. doi: 10.3389/fncel.2015.00460
46. Russo M, Crupi D, Naro A, Avanzino L, Buccafusca M, Dattola V, et al. Fatigue in patients with multiple sclerosis: from movement preparation to motor execution. *J Neurol Sci.* (2015) 351:52–7. doi: 10.1016/j.jns.2015.02.031
47. Chaves AR, Devasahayam AJ, Kelly LP, Pretty RW, Ploughman M. Exercise-induced brain excitability changes in progressive multiple sclerosis: a pilot study. *J Neurol Phys Ther.* (2020) 44:132–44. doi: 10.1097/NPT.0000000000000308
48. Ferguson B. *ACSM's Guidelines for Exercise Testing and Prescription.* 9th ed. In Pescatello L, Arena R, Riebe D, Thompson PD, editors. Philadelphia, PA: Lippincott, Williams and Wilkins (2014). Philidelphia, PA (2014).
49. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol.* (1989) 46:1121–3. doi: 10.1001/archneur.1989.00520460115022
50. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. *Clin Infect Dis.* (1994) 18(Suppl. 1):S79–83. doi: 10.1093/clinids/18.Supplement_1.S79
51. Devasahayam AJ, Chaves AR, Lasisi WO, Curtis ME, Wadden KP, Kelly LP, et al. Vigorous cool room treadmill training to improve walking ability in people with multiple sclerosis who use ambulatory assistive devices: a feasibility study. *BMC Neurol.* (2020) 20:33. doi: 10.1186/s12883-020-1611-0
52. Bredin SS, Gledhill N, Jamnik VK, Warburton DE. PAR-Q+ and ePARmed-X+: new risk stratification and physical activity clearance strategy for physicians and patients alike. *Can Fam Phys.* (2013) 59:273–7.
53. Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol.* (2009) 120:2008–39. doi: 10.1016/j.clinph.2009.08.016
54. IAE A. *Duel Energy X-Ray Absorptiometry for Bone Mineral Density and Body Composition Assessment.* Vienna: International Atomic Energy Agency (2011).
55. Kelly LP, Devasahayam AJ, Chaves AR, Wallack EM, McCarthy J, Basset FA, et al. Intensifying functional task practice to meet aerobic training guidelines in stroke survivors. *Front Physiol.* (2017) 8:809. doi: 10.3389/fphys.2017.00809
56. Krachler B, Savonen K, Komulainen P, Hassinen M, Lakka TA, Rauramaa R. Cardiopulmonary fitness is a function of lean mass, not total body weight: the DR's EXTRA study. *Eur J Prev Cardiol.* (2015) 22:1171–9. doi: 10.1177/2047487314557962
57. Learmonth YC, Dlugonski D, Pilutti LA, Sandroff BM, Klaren R, Motl RW. Psychometric properties of the fatigue severity scale and the modified fatigue impact scale. *J Neurol Sci.* (2013) 331:102–7. doi: 10.1016/j.jns.2013.05.023
58. Di Lazzaro V, Oliviero A, Saturno E, Pilato F, Insola A, Mazzone P, et al. The effect on corticospinal volleys of reversing the direction of current induced in the motor cortex by transcranial magnetic stimulation. *Exp Brain Res.* (2001) 138:268–73. doi: 10.1007/s002210100722
59. Singh AM, Duncan RE, Neva JL, Staines WR. Aerobic exercise modulates intracortical inhibition and facilitation in a nonexercised upper limb muscle. *BMC Sports Sci Med Rehabil.* (2014) 6:23. doi: 10.1186/2052-1847-6-23
60. Walsh JA, Stapley PJ, Shemmell JBH, Lepers R, McAndrew DJ. Global corticospinal excitability as assessed in a non-exercised upper limb muscle compared between concentric and eccentric modes of leg cycling. *Sci Rep.* (2019) 9:19212. doi: 10.1038/s41598-019-55858-5
61. Collins DL, Neelin P, Peters TM, Evans AC. Automatic 3D intersubject registration of MR volumetric data in standardized talairach space. *J Comput Assist Tomogr.* (1994) 18:192–205. doi: 10.1097/00004728-199403000-00005
62. McGregor KM, Carpenter H, Kleim E, Sudhyadhom A, White KD, Butler AJ, et al. Motor map reliability and aging: a TMS/fMRI study. *Exp Brain Res.* (2012) 219:97–106. doi: 10.1007/s00221-012-3070-3
63. Liepert J, Weiss T, Meissner W, Steinrücke K, Weiller C. Exercise-induced changes of motor excitability with and without sensory block. *Brain Res.* (2004) 1003:68–76. doi: 10.1016/j.brainres.2003.12.039
64. Groppa S, Oliviero A, Eisen A, Quartarone A, Cohen LG, Mall V, et al. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol.* (2012) 123:858–82. doi: 10.1016/j.clinph.2012.01.010
65. Jørgensen LM, Nielsen JE, Ravnborg M. MEP recruitment curves in multiple sclerosis and hereditary spastic paraplegia. *J Neurol Sci.* (2005) 237:25–9. doi: 10.1016/j.jns.2005.05.002
66. Thirugnanasambandam N, Khera R, Wang H, Kukke SN, Hallett M. Distinct interneuronal networks influence excitability of the surround during movement initiation. *J Neurophysiol.* (2015) 114:1102–8. doi: 10.1152/jn.00791.2014
67. Potter-Baker KA, Varnerin NM, Cunningham DA, Roelle SM, Sankarasubramanian V, Bonnett CE, et al. Influence of corticospinal tracts from higher order motor cortices on recruitment curve properties in stroke. *Front Neurosci.* (2016) 10:79. doi: 10.3389/fnins.2016.00079
68. Burke S. Missing values, outliers, robust statistics & non-parametric methods. *Sci Data Manage.* (1998) 1:32–8.
69. Friedman M. The use of ranks to avoid the assumption of normality implicit in the analysis of variance. *J Am Stat Assoc.* (1937) 32:675–701. doi: 10.1080/01621459.1937.10503522
70. Sheskin DJ. *Handbook of Parametric and Nonparametric Statistical Procedures*. 3rd ed. Boca Raton, FL: CRC Press (2003)
71. Di Pino G, Pellegrino G, Assenza G, Capone F, Ferreri F, Formica D, et al. Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat Rev Neurol.* (2014) 10:597–608. doi: 10.1038/nrneurol.2014.162
72. Forsyth JK, Bachman P, Mathalon DH, Roach BJ, Asarnow RF. Augmenting NMDA receptor signaling boosts experience-dependent neuroplasticity in the adult human brain. *Proc Natl Acad Sci USA.* (2015) 112:15331. doi: 10.1073/pnas.1509262112
73. Hunt DL, Castillo PE. Synaptic plasticity of NMDA receptors: mechanisms and functional implications. *Curr Opin Neurobiol.* (2012) 22:496–508. doi: 10.1016/j.conb.2012.01.007
74. Silasi G, Murphy TH. Stroke and the connectome: how connectivity guides therapeutic intervention. *Neuron.* (2014) 83:1354–68. doi: 10.1016/j.neuron.2014.08.052
75. Mori F, Ljoka C, Magni E, Codecà C, Kusayanagi H, Monteleone F, et al. Transcranial magnetic stimulation primes the effects of exercise therapy in multiple sclerosis. *J Neurol.* (2011) 258:1281–7. doi: 10.1007/s00415-011-5924-1
76. Palm U, Ayache SS, Padberg F, Lefaucheur JP. Non-invasive brain stimulation therapy in multiple sclerosis: a review of tDCS, rTMS and ECT results. *Brain Stimul.* (2014) 7:849–54. doi: 10.1016/j.brs.2014.09.014

77. Cirillo J, Lavender AP, Ridding MC, Semmler JG. Motor cortex plasticity induced by paired associative stimulation is enhanced in physically active individuals. *J Physiol.* (2009) 587:5831–42. doi: 10.1113/jphysiol.2009.181834
78. Triggs WJ, Calvanio R, Levine M. Transcranial magnetic stimulation reveals a hemispheric asymmetry correlate of intermanual differences in motor performance. *Neuropsychologia.* (1997) 35:1355–63. doi: 10.1016/S0028-3932(97)00077-8
79. Yacyshyn AF, Woo EJ, Price MC, McNeil CJ. Motoneuron responsiveness to corticospinal tract stimulation during the silent period induced by transcranial magnetic stimulation. *Exp Brain Res.* (2016) 234:3457–63. doi: 10.1007/s00221-016-4742-1
80. Mott DD, Lewis DV. The pharmacology and function of central GABAB receptors. *Int Rev Neurobiol.* (1994) 36:97–223. doi: 10.1016/S0074-7742(08)60304-9
81. Fernandez F, Garner CC. Over-inhibition: a model for developmental intellectual disability. *Trends Neurosci.* (2007) 30:497–503. doi: 10.1016/j.tins.2007.07.005
82. Stagg CJ, Bachtiar V, Amadi U, Gudberg CA, Ilie AS, Sampaio-Baptista C, et al. Local GABA concentration is related to network-level resting functional connectivity. *eLife.* (2014) 3:e01465. doi: 10.7554/eLife.01465
83. Singh AM, Staines WR. The effects of acute aerobic exercise on the primary motor cortex. *J Motor Behav.* (2015) 47:328–39. doi: 10.1080/00222895.2014.983450
84. Smith AE, Goldsworthy MR, Garside T, Wood FM, Ridding MC. The influence of a single bout of aerobic exercise on short-interval intracortical excitability. *Exp Brain Res.* (2014) 232:1875–82. doi: 10.1007/s00221-014-3879-z
85. Mang CS, Snow NJ, Campbell KL, Ross CJ, Boyd LA. A single bout of high-intensity aerobic exercise facilitates response to paired associative stimulation and promotes sequence-specific implicit motor learning. *J Appl Physiol.* (2014) 117:1325–36. doi: 10.1152/japplphysiol.00498.2014
86. Nepveu JF, Thiel A, Tang A, Fung J, Lundbye-Jensen J, Boyd LA, et al. A single bout of high-intensity interval training improves motor skill retention in individuals with stroke. *Neurorehabil Neural Repair.* (2017) 31:726–35. doi: 10.1177/1545968317718269
87. Sullivan KJ, Brown DA, Klassen T, Mulroy S, Ge T, Azen SP, et al. Effects of task-specific locomotor and strength training in adults who were ambulatory after stroke: results of the STEPS randomized clinical trial. *Phys Ther.* (2007) 87:1580–602. doi: 10.2522/ptj.20060310
88. Visintin M, Barbeau H, Korner-Bitensky N, Mayo NE. A new approach to retrain gait in stroke patients through body weight support and treadmill stimulation. *Stroke.* (1998) 29:1122–8. doi: 10.1161/01.STR.29.6.1122
89. Ziemann U, Muellbacher W, Hallett M, Cohen LG. Modulation of practice-dependent plasticity in human motor cortex. *Brain.* (2001) 124:1171–81. doi: 10.1093/brain/124.6.1171
90. Santarnecchi E, Rossi S, Bartalini S, Cincotta M, Giovannelli F, Tatti E, et al. Neurophysiological correlates of central fatigue in healthy subjects and multiple sclerosis patients before and after treatment with amantadine. *Neural Plast.* (2015) 2015:616242. doi: 10.1155/2015/616242
91. Vucic S, Burke T, Lenton K, Ramanathan S, Gomes L, Yannikas C, et al. Cortical dysfunction underlies disability in multiple sclerosis. *Mult Scler.* (2012) 18:425–32. doi: 10.1177/1352458511424308
92. Nantes JC, Zhong J, Holmes SA, Narayanan S, Lapierre Y, Koski L. Cortical damage and disability in multiple sclerosis: relation to intracortical inhibition and facilitation. *Brain Stimul.* (2016) 9:566–73. doi: 10.1016/j.brs.2016.01.003
93. Mango D, Nisticò R, Furlan R, Finardi A, Centonze D, Mori F. PDGF modulates synaptic excitability and short-latency afferent inhibition in multiple sclerosis. *Neurochem Res.* (2019) 44:726–33. doi: 10.1007/s11064-018-2484-0
94. Mori F, Nisticò R, Nicoletti CG, Zagaglia S, Mandolesi G, Piccinin S, et al. RANTES correlates with inflammatory activity and synaptic excitability in multiple sclerosis. *Mult Scler.* (2016) 22:1405–12. doi: 10.1177/1352458515621796
95. Wirsching I, Buttmann M, Odorfer T, Volkmann J, Classen J, Zeller D. Altered motor plasticity in an acute relapse of multiple sclerosis. *Eur J Neurosci.* (2018) 47:251–7. doi: 10.1111/ejn.13818
96. Fisher BE, Wu AD, Salem GJ, Song J, Lin CH, Yip J, et al. The effect of exercise training in improving motor performance and corticomotor excitability in people with early Parkinson's disease. *Arch Phys Med Rehabil.* (2008) 89:1221–9. doi: 10.1016/j.apmr.2008.01.013
97. Lefaucheur J-P. Motor cortex dysfunction revealed by cortical excitability studies in Parkinson's disease: influence of antiparkinsonian treatment and cortical stimulation. *Clin Neurophysiol.* (2005) 116:244–53. doi: 10.1016/j.clinph.2004.11.017
98. Thomas SL, Gorassini MA. Increases in corticospinal tract function by treadmill training after incomplete spinal cord injury. *J Neurophysiol.* (2005) 94:2844–55. doi: 10.1152/jn.00532.2005
99. Coote S, Uszynski M, Herring MP, Hayes S, Scarrott C, Newell J, et al. Effect of exercising at minimum recommendations of the multiple sclerosis exercise guideline combined with structured education or attention control education - secondary results of the step it up randomised controlled trial. *BMC Neurol.* (2017) 17:119. doi: 10.1186/s12883-017-0898-y
100. Pilutti LA, Paulseth JE, Dove C, Jiang S, Rathbone MP, Hicks AL. Exercise training in progressive multiple sclerosis: a comparison of recumbent stepping and body weight-supported treadmill training. *Int J MS Care.* (2016) 18:221–9. doi: 10.7224/1537-2073.2015-067
101. Créange A, Lefaucheur JP, Balleyguier MO, Galactéros F. Iron depletion induced by bloodletting and followed by rhEPO administration as a therapeutic strategy in progressive multiple sclerosis: a pilot, open-label study with neurophysiological measurements. *Neurophysiol Clin.* (2013) 43:303–12. doi: 10.1016/j.neucli.2013.09.004
102. Gruet M. Fatigue in chronic respiratory diseases: theoretical framework and implications for real-life performance and rehabilitation. *Front Physiol.* (2018) 9:1285. doi: 10.3389/fphys.2018.01285
103. Chang WH, Fried PJ, Saxena S, Jannati A, Gomes-Osman J, Kim YH, et al. Optimal number of pulses as outcome measures of neuronavigated transcranial magnetic stimulation. *Clin Neurophysiol.* (2016) 127:2892–7. doi: 10.1016/j.clinph.2016.04.001

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

frontiers in Neurology

PERSPECTIVE
published: 07 July 2020
doi: 10.3389/fneur.2020.00566

Inflammation and Corticospinal Functioning in Multiple Sclerosis: A TMS Perspective

Mario Stampanoni Bassi[1], *Fabio Buttari*[1], *Luana Gilio*[1], *Nicla De Paolis*[1], *Diego Fresegna*[2], *Diego Centonze*[1,3]* *and Ennio Iezzi*[1]

[1] *Unit of Neurology & Neurorehabilitation, IRCCS Neuromed, Pozzilli, Italy,* [2] *Laboratory of Synaptic Immunopathology, IRCCS San Raffaele Pisana, Rome, Italy,* [3] *Laboratory of Synaptic Immunopathology, Department of Systems Medicine, Tor Vergata University, Rome, Italy*

Transcranial magnetic stimulation (TMS) has been employed in multiple sclerosis (MS) to assess the integrity of the corticospinal tract and the corpus callosum and to explore some physiological properties of the motor cortex. Specific alterations of TMS measures have been strongly associated to different pathophysiological mechanisms, particularly to demyelination and neuronal loss. Moreover, TMS has contributed to investigate the neurophysiological basis of MS symptoms, particularly those not completely explained by conventional structural damage, such as fatigue. However, variability existing between studies suggests that alternative mechanisms should be involved. Knowledge of MS pathophysiology has been enriched by experimental studies in animal models (i.e., experimental autoimmune encephalomyelitis) demonstrating that inflammation alters synaptic transmission, promoting hyperexcitability and neuronal damage. Accordingly, TMS studies have demonstrated an imbalance between cortical excitation and inhibition in MS. In particular, cerebrospinal fluid concentrations of different proinflammatory and anti-inflammatory molecules have been associated to corticospinal hyperexcitability, highlighting that inflammatory synaptopathy may represent a key pathophysiological mechanism in MS. In this perspective article, we discuss whether corticospinal excitability alterations assessed with TMS in MS patients could be useful to explain the pathophysiological correlates and their relationships with specific MS clinical characteristics and symptoms. Furthermore, we discuss evidence indicating that, in MS patients, inflammatory synaptopathy could be present since the early phases, could specifically characterize relapses, and could progressively increase during the disease course.

Keywords: Transcranial magnetic stimulation (TMS), multiple sclerosis (MS), inflammation, synaptic transmission, cytokines

OPEN ACCESS

Edited by:
Ulrich Palm,
Medical Park Chiemseeblick, Germany

Reviewed by:
Yasuo Terao,
Kyorin University, Japan
Sasa Filipovic,
University of Belgrade, Serbia

***Correspondence:**
Diego Centonze
centonze@uniroma2.it

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *31 January 2020*
Accepted: *19 May 2020*
Published: *07 July 2020*

Citation:
Stampanoni Bassi M, Buttari F, Gilio L, De Paolis N, Fresegna D, Centonze D and Iezzi E (2020) Inflammation and Corticospinal Functioning in Multiple Sclerosis: A TMS Perspective. Front. Neurol. 11:566. doi: 10.3389/fneur.2020.00566

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory immune-mediated disease of the central nervous system (CNS) with white matter demyelinating lesions and chronic diffuse neuronal degeneration, causing variable and unpredictable clinical manifestations and disease course.

Transcranial magnetic stimulation (TMS) is a neurophysiological technique that exploits the principles of electromagnetic induction. A coil of wire, connected to an electric pulse generator

and placed over the scalp, produces a strong magnetic pulse of very short duration able to penetrate through the intact skull, inducing an electric current in the underlying neural tissue noninvasively and almost painlessly (1). The induced electric current mainly flows tangentially to the brain surface, preferentially activating cortical fibers oriented in parallel to the electric field (2). When applied over the primary motor cortex (M1), TMS excites the corticospinal system, eliciting multiple descending volleys, which reflect both the direct activation of cortical motor axons (D-waves) and the indirect, trans-synaptic activation of motor cortical neurons (I-waves) (3, 4). The recruitment of different combinations of D- and I-waves depends on the stimulus intensity, pulse configuration, coil shape, and orientation (5–9). In particular, with a posterior-to-anterior induced current flow, TMS at lower intensities evokes I-waves, whereas at higher intensities also D-waves occur (10). These descending activities can be recorded in contralateral target muscles as motor-evoked potentials (MEPs).

TMS is used in the clinical context of MS together with multimodal evoked potentials (i.e., visual and somatosensory) as a useful tool to detect subclinical involvement of the corresponding functional system with the aim to help early diagnosis (11). TMS alterations have also been correlated to demyelinating damage and neuronal degeneration in different MS phenotypes. For example, slowed central motor conduction time (CMTC) and reduced MEP amplitude can indicate axonal depletion or even extreme asynchrony of the multiple descending volleys to spinal motoneurons due to conduction blocks in the myelinated fibers along the corticospinal tracts (12, 13).

Experimental evidence from studies in animal models and in patients with MS suggests that inflammation critically affects synaptic functioning (14). Accordingly, neurophysiological alterations have been detected even in the absence of macroscopic damage, suggesting a role of additional pathological mechanisms (15). In particular, different proinflammatory and anti-inflammatory molecules can influence cortical excitability in MS (16) representing an additional cause of impaired synaptic functioning.

In this perspective article, we provide an overview of the main TMS studies exploring corticospinal excitability and connectivity alterations in MS, their pathophysiological correlates, and their relationship to clinical characteristics and symptoms. In addition, evidence from preclinical data and TMS studies, which highlight the role of inflammatory synaptopathy as a relevant pathophysiological mechanism that acts since the early phases of MS, is discussed.

TMS as a Tool to Investigate Cortical Excitability in MS

TMS can be used to assess the functionality of the corticospinal tract and the corpus callosum (CC) and to explore some physiological properties of M1. Various TMS paradigms have been designed to investigate corticospinal excitability to test excitatory and inhibitory interactions in M1 and to probe M1 connectivity **(Table 1)**. Single-pulse TMS can be used to assess simple cortical excitability measures, such as motor thresholds, to study MEP characteristics, to estimate CMCT, and to test cortical inhibition. With paired-pulse TMS, it is possible to explore specific inhibitory and excitatory circuits in M1. During paired-pulse TMS, two stimulators are connected to the same coil that delivers two consecutive pulses at variable interstimulus intervals (ISIs). In addition, TMS has been used to investigate interhemispheric effective connectivity of M1 by exploring transcallosal connections with either single or double-coil (d-c) approaches. In d-c TMS, two stimulation units, each connected to a corresponding coil, are used to target different motor cortical regions at various ISIs.

TMS as a Tool to Investigate Different Pathophysiological Mechanisms in MS

Considering the clinical impact of corticospinal system lesions, different TMS studies have shown several alterations of M1 excitability and corticospinal tract conduction in MS patients. In particular, reduced MEP amplitude (54–56), increased MEP latency (57), and duration (58) have been reported in MS patients compared with control subjects. In addition, increased RMT (55, 59) and prolonged CMCT (54, 59–62) have been frequently evidenced in patients with MS. Overall, these findings have been interpreted in the light of demyelinating conduction block and axonal damage. In fact, demyelination and conduction blocks could lead to a greater temporal dispersion of the corticospinal volleys, resulting in reduced amplitude and increased MEP duration, prolonged MEP latency, and increased CMCT. Conversely, axonal loss could be more relevant in progressive MS, being associated with higher RMT, reduced MEP amplitude, and longer CMCT (55).

Cortical inhibition tested with single-pulse TMS has documented prolonged CSP duration in RR-MS patients (57, 63). One study has shown that, in remitting patients, CSP prolongation was correlated with white matter lesion volume but not with cortical thickness (57). In progressive MS patients, reduced CSP duration correlated with lower whole-brain cortical magnetization transfer ratio (MTR), suggesting a role of cortical damage (56). Altered GABA transmission could explain the CSP alteration although alternative mechanisms have been suggested, including changes in spinal motoneuron excitability (23, 24), attentional processes (64, 65), and altered voluntary motor drive (66). In addition, reduced CSP duration after a fatiguing motor task has been reported in MS patients compared to controls (67), suggesting that additional mechanisms could also be involved.

Various studies have explored SICI in MS patients (55, 56, 68). Although some authors reported comparable SICI between RR-MS and controls (55, 57, 69), in one study it has been found that lower SICI in patients with RR-MS was correlated with reduced MTR in the hand motor cortex (56). In addition, reduced SICI and increased ICF have been reported in SP-MS patients compared with RR-MS and controls (55, 69). It has been proposed that the clinical course of progressive MS phenotypes could be characterized by a deterioration of SICI over time (70). These alterations may reflect hyperexcitability due to enhanced glutamatergic transmission and reduced inhibition, which could be particularly noticeable in progressive patients, being associated with higher disability and cortical atrophy (55).

TABLE 1 | Main TMS protocols used to explore motor cortex pathophysiology in MS patients.

SINGLE-PULSE TMS	**Motor thresholds**	Resting and active motor thresholds (RMT and AMT), tested with single-pulse TMS in resting and contracted muscles respectively, represent simple measures of the excitability of the whole corticospinal system, including the fluctuating excitability of both M1 pyramidal cells and spinal motoneurons (9) MTs are defined as the minimum intensity of M1 stimulation able to elicit MEPs in the target muscles (9) and MTs likely depend on the axonal excitability regulated by voltage-gated sodium channels and on the activity of AMPA receptors (17).
	MEP amplitude	Commonly used for testing the excitability of the whole corticospinal system. TMS activates along the corticospinal tracts a series of descending volleys with different thresholds, different conduction velocities, and intrinsic asynchrony of propagation (8, 10). Temporal dispersion is further enhanced by peripheral nerve conduction, leading to phase cancellation of motor unit action potentials (18, 19). MEP amplitude can be influenced by excitability changes occurring at cortical level, representing an important marker of synaptic activity in the motor cortex (20). Spinal motoneurons excitability also contributes to MEP amplitude. (8, 21).
	Central motor conduction time (CMCT)	Represents the time interval elapsing between the cortical stimulus and the arrival of the excitatory input to the spinal motoneurons, being a useful tool to assess the integrity of fast-conducting motor pathways in the corticospinal tract (9). CMCT evaluated with TMS includes the trans-synaptic activation of cortical motoneurons through the chain of cortical interneurons responsible for the I-waves generation. CMCT is commonly calculated by subtracting the peripheral motor conduction time from the MEPs latency (9).
	Cortical silent period (CSP)	Inhibitory phenomenon measured in contracting muscles as an interruption of the ongoing voluntary electromyographic activity. Spinal inhibitory mechanisms contribute to the first part of CSP, whereas the late part originates at cortical level (22–24) and expresses GABA-B mediated inhibition in M1 (25–27). The role of GABA-A receptors has also been suggested. In particular, GABA-A receptors could be activated by low stimulus intensity, whereas GABA-B receptors are engaged with stronger pulses (28).
PAIRED-PULSE TMS	**Short-interval intracortical inhibition (SICI)**	SICI is tested with a subthreshold conditioning stimulus followed by a suprathreshold test stimulus at an ISI of 2-5 ms (29). The conditioning stimulus suppresses the excitatory response to the subsequent suprathreshold stimulus (29, 30) depending on GABA-A receptor activity (30–32).
	Intracortical facilitation (ICF)	ICF is evaluated with a similar protocol used for SICI with longer interstimulus intervals at 7–20 ms (29, 33). ICF engages M1 circuits different from those involved in SICI with a resulting excitatory effect that combines a weak GABA-A-mediated inhibition and a predominant NMDA-mediated facilitation (33, 34).
	Short-interval intracortical facilitation (SICF)	SICF is measured with a particular paired-pulse TMS protocol that uses at very short ISIs either a conditioning suprathreshold stimulus followed by a test subthreshold stimulus (34, 35) or two near threshold pulses (36, 37) and reflects facilitatory I-wave interaction within M1 (35). Pharmacological studies have suggested that SICF is modulated by a number of neurotransmitter systems, including GABA, dopamine, noradrenaline [for reviews, see (35, 38)].
TRANSCALLOSAL CONNECTIVITY	**Ipsilateral silent period (iSP)**	Tests the inhibitory influences existing between the two M1s and is mediated by fibers passing across the corpus callosum (39–41). Refers to the suppression of ongoing voluntary electromyographic activity in hand muscles in response to a single suprathreshold pulse over the ipsilateral M1 likely mediated by GABAergic transcallosal projections (42–44).
	Interhemispheric connectivity	Interhemispheric inhibition (IHI) studied with d-c TMS, refers to the suppression of MEPs following suprathreshold conditioning stimuli given over the contralateral M1 (43, 45). IHI is mediated by excitatory inputs coming from the conditioned M1, traveling across the corpus callosum, and reaching local inhibitory synapses in the contralateral target M1 (43, 46, 47) are mediated by GABA-B (48–50). Facilitatory interhemispheric connections have also been studied between dorsal premotor cortex and contralateral M1 (51–53).

As the CC involvement represents a hallmark of MS, TMS has been specifically used to test interhemispheric connectivity in these patients. Increased iSP latency and duration have been reported in MS patients compared to controls (60–62, 71). In particular, iSP alterations found in MS have been associated to CC volume (62). One study in MS patients, combining TMS and fMRI, has demonstrated that increased ipsilateral M1 activation during the execution of a motor task was correlated with reduced iSP duration and with ultrastructural damage of the CC (61). However, prolonged iSP duration has also been associated with CMCT prolongation without significant correlations with CC abnormalities, suggesting that transcallosal inhibition could be affected by demyelination of the contralateral corticospinal tract (72). Notably, reduced IHI has also been observed in early RR-MS patients in the absence of macroscopic damage of the CC or CMCT alterations (73). Finally, one TMS study has shown that excitatory interhemispheric connectivity between premotor cortex and contralateral M1 could be reduced, irrespective of CC lesion load and in the absence of disability (53). Although the pathophysiological mechanisms underlying altered interhemispheric connectivity in MS are not fully understood, it is likely that, alternatively to CC structural damage, other

mechanisms could be involved, including reduced excitatory projections from the conditioning cortex or defective GABAergic signaling in target M1 inhibitory interneurons (48, 74).

TMS Alterations Could Be Associated With Specific MS Clinical Characteristics

Alterations of various TMS measures have been related to MS clinical characteristics. Expanded disability status scale (EDSS) score has been associated with increased RMT, altered MEPs, and prolonged CMCT and iSP duration (55, 58, 60). A positive correlation between these TMS measures and clinical scores could reflect the prevalent role of white matter lesions in the pathogenesis of these alterations, particularly of the corticospinal tract and the CC. The role of white matter disconnection has been specifically involved in cerebellar symptoms. Cerebellar tremor in MS has been associated with lacking cerebellar-M1 inhibitory connectivity tested with d-c TMS (75). In addition, cerebellar dysfunction in MS has also been associated with increased CSP duration, likely resulting from impaired cerebellar projections to M1 (63).

Altered balance between cortical excitation and inhibition in MS has been correlated with clinical severity. One study showed that prolonged CSP duration was correlated with clinical disability and predicted greater motor impairment, suggesting that increased inhibition could lessen clinical compensation, possibly interfering with plasticity (57). Moreover, one study has demonstrated that defective SICF was correlated with increased EDSS in MS patients (76). Alterations involving both inhibitory and excitatory circuits would suggest a specific role of synaptic dysfunction in addition to demyelination of white matter tracts. The finding that steroid administration in relapsing RR-MS led to motor improvement, along with reduced SICI and enhanced ICF (77), supports this hypothesis, suggesting a restored synaptic functioning within M1. Finally, it has been proposed that corticospinal excitability asymmetry between the two hemispheres could represent a marker of clinical disability, whose mechanisms are not completely elucidated and possibly involving neurodegenerative and inflammatory processes (78).

Fatigue represents a frequent and severely disabling symptom in MS patients (79). Different mechanisms have been postulated, including white matter and cortical lesions, endocrine alterations, and the influence of neuroinflammation on brain functioning (80, 81). Enhanced GABAergic activity tested with SICI and CSP has been specifically implicated in MS fatigue (82, 83). In line with the hypothesis of increased M1 inhibition in fatigued MS patients, one study has demonstrated that a fatiguing motor task was associated with increased CSP duration. Notably, unlike healthy controls, CSP alteration also involved untrained adjacent muscles, suggesting that mechanisms of cortical spreading could intervene in generating fatigue in MS (67).

Cognitive dysfunction represents an important symptom frequently underestimated in MS patients, which involves various domains, including executive functions, processing speed, and working memory. In addition to demyelination and gray matter atrophy, different pathophysiological mechanisms, including the presence of cortical lesions, impaired brain network organization, and altered synaptic functioning, have been proposed (84). Short-latency afferent inhibition (SAI), a TMS protocol exploring the efficiency of cortical cholinergic inhibitory activity mediated by peripheral somatosensory afferent inputs to M1 (85), has been used to investigate cognitive dysfunction in MS. In particular, verbal memory impairment was associated with reduced SAI that could be partly reversed by rivastigmine administration (86). Notably, these results are in line with studies demonstrating altered SAI in patients with Alzheimer's disease (87). Although mood disturbances are frequently observed in MS, correlations with TMS alterations have been scarcely investigated. One study showed that anxiety in MS patients was associated with altered inhibitory interhemispheric connectivity, highlighting the role of increased transcallosal transfer (88).

INFLAMMATORY SYNAPTOPATHY AS A LINK BETWEEN AUTOIMMUNITY AND DISEASE MANIFESTATIONS IN MS

In MS, auto-reactive T lymphocyte infiltration into the CNS and activation of resident immune cells lead to demyelinating lesions and axonal damage. Inflammatory cytokines released by immune cells play a crucial role in inducing and maintaining the inflammatory response in MS. Proinflammatory molecules promote T-helper 1 (Th1) and Th17 differentiation and lymphocyte activation and migration across the blood brain barrier (89). Accordingly, enhanced expression of various cytokines, including interleukin (IL)-1β, tumor necrosis factor (TNF), IL-6, IL-17, and interferon (IFN)-γ has been reported in animal models (i.e., experimental autoimmune encephalomyelitis, EAE) and in the perivascular infiltrates and cerebrospinal fluid (CSF) of MS patients (90–94).

In addition to their immunomodulatory activity, cytokines modulate the function of oligodendrocytes, astrocytes, and neurons (95, 96). Experimental studies have shown that inflammatory molecules specifically influence synaptic functioning, suggesting that chemokines and cytokines may represent an important communicating system in the CNS. In turn, astrocytes, endothelial cells, and neurons participate in cytokine production (97, 98), generating a neuro-immune crosstalk with crucial roles in physiological and pathological conditions (99, 100).

Preclinical Studies and Translational Models

Experimental studies have contributed to demonstrate that inflammation alters synaptic functioning (14, 101). In the striatum of EAE mice, electrophysiological recordings revealed enhanced glutamatergic transmission and excitotoxic neurodegeneration occurring since the early phases, before the onset of symptoms, and independently of demyelinating damage (14). These excitotoxic alterations were mainly caused by increased activity and expression of the a-amino-3-hydroxy-5-methyl-4-isoxazolepropionicacid (AMPA) receptor; accordingly, the administration of inhibitors of glutamate AMPA receptors ameliorated the course of EAE and reduced loss of dendritic spines (14). In the same study, TNF released by activated

microglial cells was identified as mainly responsible for these alterations as incubation of this molecule reproduced *in vitro* both altered AMPA activity and neuronal damage. Other inflammatory cytokines have been associated with synaptic hyperexcitability in EAE. The proinflammatory cytokine IL-1β induced pathologically enhanced glutamatergic transmission in the cerebellum of EAE mice, reducing glutamate reuptake by altering the expression of the glutamate-aspartate transporter/excitatory amino acid transporter 1 (GLAST/EAAT1) (102). Notably, the administration of the GLAST/EAAT1 inhibitor reproduced the synaptic modifications observed in symptomatic EAE mice (103). In addition, administration of the IL-1 receptor antagonist, a physiological inhibitor of IL-1β (104), ameliorated the course of EAE by reducing astroglia activation and restoring GLAST/EAAT1 expression (102, 105). Proinflammatory cytokines have also been consistently associated with altered inhibitory transmission in EAE mice. It has been evidenced that incubating IL-1β and TNF in mice brain slices impaired GABAergic transmission and promoted excitotoxic neuronal damage (106, 107). Accordingly, enhancing GABA signaling significantly improved the clinical symptoms of EAE, likely as a result of a direct neuroprotective effect and inhibition of inflammatory response (108).

Translational experiments confirmed that a similar subset of proinflammatory molecules mediates synaptic alterations in human MS. One study has demonstrated that the CSF collected from patients with active MRI lesions pathologically enhanced excitatory postsynaptic currents when incubated on mice brain slices, inducing glutamate-mediated neuronal damage (109). IL-1β has been identified as mainly responsible for these alterations by increasing AMPA receptor activity. Inflammation-induced synaptic alterations in MS have also been investigated using a heterologous chimeric model. T-lymphocytes isolated from the peripheral blood of RR-MS patients exacerbated the glutamatergic transmission when incubated on mice brain slices (110). In particular, only lymphocytes from patients with acute inflammation, as evidenced by the presence of gadolinium-enhancing lesions at MRI, were able to induce synaptic alterations. Notably, co-incubation with etanercept, a TNF antagonist, prevented these alterations, confirming that TNF was mainly responsible for these findings (110).

Inflammation and Corticospinal Excitability in MS

The role of inflammation on synaptic dysfunction in MS has been specifically addressed by some TMS studies. In relapsing MS patients, it has been shown to both reduce CSP duration and impair SICI compared to remitting patients (111). These results demonstrate that the relapsing phases could be characterized by cortical hyperexcitability, suggesting reduced GABAergic transmission similarly to as evidenced in animal models (106, 107). To explore the role of CSF inflammation on cortical excitability, different TMS measures have been correlated with the levels of specific proinflammatory molecules. In relapsing MS patients, elevated IL-1β signaling has been associated with increased ICF without effect on SICI (109). This finding has confirmed the main role of this molecule in altering synaptic functioning also in human MS by enhancing glutamatergic transmission (109). The involvement of this molecule in the excitotoxic degeneration has also been suggested by clinical studies, showing that CSF IL-1β detectability during remissions predicted greater prospective disability and neurodegeneration (112). Other inflammatory mediators have also been associated to altered synaptic transmission in relapsing MS patients. Regulated upon activation, normal T-cell expressed and secreted (RANTES) is a proinflammatory molecule regulating the leukocyte chemotaxis (113). Increased RANTES concentrations have been found in the CSF of MS patients with acute inflammation and correlated with both reduced SICI and increased ICF (114). Finally, incubating this molecule on mice hippocampal slices promoted hyperexcitability and excitotoxicity (114), confirming the role of RANTES as a central regulator of glutamatergic transmission (113).

Overall, these results indicate that exacerbated CSF inflammation negatively influences the disease course of MS, promoting synaptic hyperexcitability and neuronal damage. It has been proposed that neurodegeneration in progressive MS phenotypes could also result from inflammation-driven synaptic alterations. In fact, reduced SICI and enhanced ICF have been reported in SP-MS patients and have been related to enhanced disability (55). These findings suggest that glutamatergic excitotoxic damage could characterize the progressive MS phenotypes as demonstrated by *in vitro* studies showing hyperexcitability and enhanced neuronal damage induced by CSF collected from progressive MS patients, mediated by TNF (115). Conversely, anti-inflammatory cytokines, including IL-10 and IL-13, and neurotrophic factors may exert protective effects, reducing neurodegeneration and promoting a better disease course in EAE and MS (116–119). TMS studies have confirmed that anti-inflammatory molecules could reduce the synaptic alterations in MS. Accordingly, in RR-MS patients, the CSF levels of the anti-inflammatory molecule IL-13 have been associated with increased SICI, possibly contributing to restored inhibitory synaptic activity and limiting the impact of excitotoxicity. Notably, IL-13 CSF levels were also associated with reduced measures of neuronal and axonal damage and with increased amyloid-beta CSF concentrations, suggesting a protective role of this cytokine in MS (120).

CONCLUSIONS AND FUTURE PERSPECTIVES

Various TMS protocols have been used to characterize the neurophysiological correlates of specific pathophysiological mechanisms, such as demyelination and neuronal loss, in different disease phases and phenotypes. These studies have contributed to better defining the neurophysiological basis of specific MS symptoms, particularly those not completely explained by conventional structural damage measures, such as fatigue and cognitive deficits. Alterations of corticospinal excitability and corticospinal tract conduction have been clearly linked to both demyelinating blocks and axonal damage. MEP latency and amplitude are the most frequently altered TMS measures in MS and have been consistently associated

with disability, representing useful tools in clinical settings. Although TMS studies investigating intracortical excitability and effective connectivity have shown some association with specific pathophysiological mechanisms or disease phenotypes, some discrepancies suggest that alternative mechanisms should be involved.

Evidence from experimental studies suggests that inflammatory synaptopathy could represent an independent cause of synaptic dysfunction with important implications on disease course and prognosis. Inflammation, altering corticospinal excitability and connectivity in MS patients, could contribute to better explain the variability of TMS findings. Experimental models have clearly shown that inflammation exacerbates synaptic hyperexcitability, and TMS studies have confirmed an imbalance between excitatory and inhibitory transmission in MS patients. Hence, inflammation-driven synaptic hyperexcitability could be present since the early phases, could specifically characterize MS relapses, and could progressively increase during the disease course.

Having in mind that TMS measures represent the resulting effect of different anatomic and physiological factors, it is difficult to identify the contribution of specific mechanisms to TMS alterations seen in MS patients. Therefore, when cortical excitability measures are used to investigate MS pathophysiology, the role of specific confounding factors, including disease activity and phenotypes, ongoing therapies, and symptoms, such as fatigue, should be carefully considered. Further studies conducted in specific populations, such as patients with clinically isolated syndrome or with progressive MS, or combining TMS with structural and/or functional imaging data, could help to shed light on the specific role of demyelination, atrophy, and inflammation.

AUTHOR CONTRIBUTIONS

MS, EI, and DC contributed conception and design of the study, MS and EI wrote the first draft of the manuscript, FB, LG, ND, and DF wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

FUNDING

This research was supported by FISM-Fondazione Italiana Sclerosi Multipla-cod. 2019/S/1 to DC; by the Italian Ministry of Health (Ricerca corrente-IRCCS Neuromed to DC; Ricerca Finalizzata 2018, RF-2018-12366144 to DC; Ricerca Finalizzata 2018, GR-2018-12366154 to FB) and by 5 × 1000 grant to IRCCS Neuromed. 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, or in the decision to publish the results.

REFERENCES

1. Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. *Lancet.* (1985) 1:1106–07.
2. Hallett M. Transcranial magnetic stimulation and the human brain. *Nature.* (2000) 406:147–50. doi: 10.1038/35018000
3. Amassian VE, Stewart M, Quirk GJ, Rosenthal JL. Physiological basis of motor effects of a transient stimulus to cerebral cortex. *Neurosurgery.* (1987) 20:74-93.
4. Shimazu H, Maier MA, Cerri G, Kirkwood PA, Lemon RN. Macaque ventral premotor cortex exerts powerful facilitation of motor cortex outputs to upper limb motoneurons. *J Neurosci.* (2004) 24:1200–11. doi: 10.1523/JNEUROSCI.4731-03.2004
5. Nakamura H, Kitagawa H, Kawaguchi Y, Tsuji H. Direct and indirect activation of human corticospinal neurons by transcranial magnetic and electrical stimulation. *Neurosci Lett.* (1996) 210:45–8. doi: 10.1016/0304-3940(96)12659-8
6. Di Lazzaro V, Oliviero A, Mazzone P, Insola A, Pilato F, Saturno E, et al. Comparison of descending volleys evoked by monophasic and biphasic magnetic stimulation of the motor cortex in conscious humans. *Exp Brain Res.* (2001) 141:121–7. doi: 10.1007/s002210100863
7. Kammer T, Beck S, Thielscher A, Laubis-Herrmann U, Topka H. Motor thresholds in humans: a transcranial magnetic stimulation study comparing different pulse waveforms, current directions and stimulator types. *Clin Neurophysiol.* (2001) 112:250-58. doi: 10.1016/s1388-2457(00)00513-7
8. Groppa S, Oliviero A, Eisen A, Quartarone A, Cohen LG, Mall V, et al. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol.* (2012) 123:858-882. doi: 10.1016/j.clinph.2012.01.010
9. Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin Neurophysiol.* (2015) 126:1071–107. doi: 10.1016/j.clinph.2015.02.001
10. Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Mazzone P, et al. The physiological basis of transcranial motor cortex stimulation in conscious humans. *Clin Neurophysiol.* (2004) 115:255-66. doi: 10.1016/j.clinph.2003.10.009
11. Ziemann U, Wahl M, Hattingen E, Tumani H. Development of biomarkers for multiple sclerosis as a neurodegenerative disorder. *Prog Neurobiol.* (2011) 95:670–85. doi: 10.1016/j.pneurobio.2011.04.007
12. Hess CW, Mills KR, Murray NM. Measurement of central motor conduction in multiple sclerosis by magnetic brain stimulation. *Lancet.* (1986) 2:355–8. doi: 10.1016/S0140-6736(86)90050-4
13. Hess CW, Mills KR, Murray NM, Schriefer TN. Magnetic brain stimulation: central motor conduction studies in multiple sclerosis. *Ann Neurol.* (1987) 22:744–52. doi: 10.1002/ana.410220611
14. Centonze D, Muzio L, Rossi S, Cavasinni F, De Chiara V, Bergami A, et al. Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. *J Neurosci.* (2009) 29:3442-52. doi: 10.1523/JNEUROSCI.5804-08.2009
15. Zeis T, Graumann U, Reynolds R, Schaeren-Wiemers N. Normal-appearing white matter in multiple sclerosis is in a subtle balance between inflammation and neuroprotection. *Brain.* (2008) 131:288-303. doi: 10.1093/brain/awm291
16. Stampanoni Bassi M, Mori F, Buttari F, Marfia GA, Sancesario A, Centonze D, et al. Neurophysiology of synaptic functioning in multiple sclerosis. *Clin Neurophysiol.* (2017) 128:1148-57. Review. doi: 10.1016/j.clinph.2017.04.006
17. Ziemann U, Reis J, Schwenkreis P, Rosanova M, Strafella A, Badawy R, et al. TMS and drugs revisited 2014. *Clin Neurophysiol.* (2015) 126:1847-68. doi: 10.1016/j.clinph.2014.08.028
18. Rossini PM, Marciani MG, Caramia M, Roma V, Zarola F. Nervous propagation along 'central' motor pathways in intact man: characteristics of motor responses to 'bifocal' and 'unifocal' spine and scalp non-invasive stimulation. *Electroencephalogr Clin Neurophysiol.* (1985) 61:272–86. doi: 10.1016/0013-4694(85)91094-6

19. Magistris MR, Rosler KM, Truffert A, Myers JP. Transcranial stimulation excites virtually all motor neurons supplying the target muscle. A demonstration and a method improving the study of motor evoked potentials. *Brain*. (1998) 121:437–50. doi: 10.1093/brain/121.3.437
20. Ferreri F, Vecchio F, Ponzo D, Pasqualetti P, Rossini PM. Time-varying coupling of EEG oscillations predicts excitability fluctuations in the primary motor cortex as reflected by motor evoked potentials amplitude: an EEG–TMS study. *Hum Brain Mapp*. (2014) 35:1969–80. doi: 10.1002/hbm.22306
21. Taylor JL. Stimulation at the cervicomedullary junction in human subjects. *J Electromyogr Kinesiol.* (2006) 16:215-23. doi: 10.1016/j.jelekin.2005.07.001
22. Fuhr P, Agostino R, Hallett M. Spinal motor neuron excitability during the silent period after cortical stimulation. *Electroencephalogr Clin Neurophysiol.* (1991) 81:257–62. doi: 10.1016/0168-5597(91)90011-l
23. Inghilleri M, Berardelli A, Cruccu G, Manfredi M. Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. *J Physiol.* (1993) 466:521-34.
24. Ziemann U, Netz J, Szelenyi A, Hömberg V. Spinal and supraspinal mechanisms contribute to the silent period in the contracting soleus muscle after transcranial magnetic stimulation of human motor cortex. *Neurosci Lett.* (1993) 156:167–71. doi: 10.1016/0304-3940(93)90464-v
25. Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *J Physiol.* (1999) 517(Pt 2):591-7. doi: 10.1111/j.1469-7793.1999.0591t.x
26. Pierantozzi M, Marciani MG, Palmieri MG, Brusa L, Galati S, Caramia MD, et al. Effect of Vigabatrin on motor responses to transcranial magnetic stimulation: an effective tool to investigate in vivo GABAergic cortical inhibition in humans. *Brain Res.* (2004) 1028:1–8. doi: 10.1016/j.brainres.2004.06.009
27. Siebner HR, Dressnandt J, Auer C, Conrad B. Continuous intrathecal baclofen infusions induced a marked increase of the transcranially evoked silent period in a patient with generalized dystonia. *Muscle Nerve.* (1998) 21:1209–12. doi: 10.1002/(sici)1097-4598(199809)21:9<1209::aid-mus15>3.0.co;2-m
28. Kimiskidis VK, Papagiannopoulos S, Kazis DA, Sotirakoglou K, Vasiliadis G, Zara F, et al. Lorazepam-induced effects on silent period and corticomotor excitability. *Exp Brain Res.* (2006) 173:603-11. doi: 10.1007/s00221-006-0402-1
29. Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. *J Physiol.* (1993) 471:501–19. doi: 10.1113/jphysiol.1993.sp019912
30. Ilic TV, Meintzschel F, Cleff U, Ruge D, Kessler KR, Ziemann U. Short-interval paired-pulse inhibition and facilitation of human motor cortex: the dimension of stimulus intensity. *J Physiol.* (2002) 545:153–67. doi: 10.1113/jphysiol.2002.030122
31. Di Lazzaro V, Oliviero A, Meglio M, Cioni B, Tamburrini G, Tonali P, et al. Direct demonstration of the effect of lorazepam on the excitability of the motor cortex. *Clin Neurophysiol.* (2000). 111:794–99. doi: 10.1016/s1388-2457(99)00314-4
32. Ziemann U, Lönnecker S, Steinhoff BJ, Paulus W. The effect of lorazepam on the motor cortical excitability in man. *Exp Brain Res.* (1996) 109:127-35. doi: 10.1007/bf00228633
33. Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. *J Physiol.* (1996) 496:873–81. doi: 10.1113/jphysiol.1996.sp021734
34. Hanajima R, Ugawa Y, Terao Y, Sakai K, Furubayashi T, Machii K, et al. Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves. *J Physiol.* (1998) 509:607-18. doi: 10.1111/j.1469-7793.1998.607bn.x
35. Ziemann U, Tergau F, Wassermann EM, Wischer S, Hildebrandt J, Paulus W. Demonstration of facilitatory I-wave interaction in the human motor cortex by paired transcranial magnetic stimulation. *J Physiol.* (1998) 511:181–90. doi: 10.1111/j.1469-7793.1998.181bi.x
36. Tokimura H, Ridding MC, Tokimura Y, Amassian VE, Rothwell JC. Short latency facilitation between pairs of threshold magnetic stimuli applied to human motor cortex. Electroencephalogr *Clin Neurophysiol.* (1996) 101:263-72.
37. Di Lazzaro V, Rothwell JC, Oliviero A, Profice P, Insola A, Mazzone P, et al. Intracortical origin of the short latency facilitation produced by pairs of threshold magnetic stimuli applied to human motor cortex. *Exp Brain Res.* (1999) 129:494-499. doi: 10.1007/s002210050919
38. Di Lazzaro and Ziemann U. The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. *Front Neural Circuits.* (2013) 7:18. doi: 10.3389/fncir.2013.00018
39. Boroojerdi B, Diefenbach K, Ferbert A. Transcallosal inhibition in cortical and subcortical cerebral vascular lesions. *J Neurol Sci.* (1996) 144:160-70. doi: 10.1016/s0022-510x(96)00222-5
40. Meyer BU, Röricht S, Woiciechowsky C. Topography of fibers in the human corpus callosum mediating interhemispheric inhibition between the motor cortices. *Ann Neurol.* (1998) 43:360-9. doi: 10.1002/ana.410430314
41. Heinen F, Glocker FX, Fietzek U, Meyer BU, Lücking CH, Korinthenberg R. Absence of transcallosal inhibition following focal magnetic stimulation in preschool children. *Ann Neurol.* (1998) 43:608-12. doi: 10.1002/ana.410430508
42. Wassermann EM, Fuhr P, Cohen LG, Hallett M. Effects of transcranial magnetic stimulation on ipsilateral muscles. *Neurology.* (1991) 41:1795-9. doi: 10.1212/wnl.41.11.1795
43. Ferbert A, Priori A, Rothwell JC, Day BL, Colebatch JG, Marsden CD. Interhemispheric inhibition of the human motor cortex. *J Physiol.* (1992) 453:525-46. https://doi.org/10.1113/jphysiol.1992.sp019243
44. Meyer BU, Röricht S, Gräfin von Einsiedel H, Kruggel F, Weindl A. Inhibitory and excitatory interhemispheric transfers between motor cortical areas in normal humans and patients with abnormalities of the corpus callosum. *Brain.* (1995) 118:429-40. doi: 10.1093/brain/118.2.429
45. Uehara K, Morishita T, Kubota S, Hirano M, Funase K. Functional difference in short- and long-latency interhemispheric inhibitions from active to resting hemisphere during a unilateral muscle contraction. *J Neurophysiol.* (2014) 111:17-25. doi: 10.1152/jn.00494.2013
46. Wahl M, Lauterbach-Soon B, Hattingen E, Jung P, Singer O, Volz S, et al. Human motor corpus callosum: topography, somatotopy, and link between microstructure and function. *J Neurosci.* (2007) 27:12132-38. doi: 10.1523/JNEUROSCI.2320-07.2007
47. Ni Z, Gunraj C, Nelson AJ, Yeh IJ, Castillo G, Hoque T, et al. Two phases of interhemispheric inhibition between motor related cortical areas and the primary motor cortex in human. *Cereb Cortex.* (2009) 19:1654-65. doi: 10.1093/cercor/bhn201
48. Kukaswadia S, Wagle-Shukla A, Morgante F, Gunraj C, Chen R. Interactions between long latency afferent inhibition and interhemispheric inhibitions in the human motor cortex. *J Physiol.* (2005) 563:915-24. doi: 10.1113/jphysiol.2004.080010
49. Radhu N, Ravindran LN, Levinson AJ, Daskalakis ZJ. Inhibition of the cortex using transcranial magnetic stimulation in psychiatric populations: current and future directions. *J Psychiatry Neurosci.* (2012) 37:369-78. Review. doi: 10.1503/jpn.120003
50. Irlbacher K, Brocke J, Mechow JV, Brandt SA. Effects of GABA(A) and GABA(B) agonists on interhemispheric inhibition in man. *Clin Neurophysiol.* (2007) 118:308-16. doi: 10.1016/j.clinph.2006.09.023
51. Bäumer T, Bock F, Koch G, Lange R, Rothwell JC, Siebner HR, Münchau A. Magnetic stimulation of human premotor or motor cortex produces interhemispheric facilitation through distinct pathways. *J Physiol.* (2006) 572:857-68.
52. Mochizuki H, Huang YZ, Rothwell JC. Interhemispheric interaction between human dorsal premotor and contralateral primary motor cortex. *J Physiol.* (2004) 561:331–8. doi: 10.1113/jphysiol.2004.072843
53. Codecà C, Mori F, Kusayanagi H, Monteleone F, Boffa L, Paolillo A, et al. Differential patterns of interhemispheric functional disconnection in mild and advanced multiple sclerosis. *Mult Scler.* (2010) 16:1308-16. doi: 10.1177/1352458510376957
54. Kale N, Agaoglu J, Tanik O. Electrophysiological and clinical correlates of corpus callosum atrophy in patients with multiple sclerosis. *Neurol Res.* (2010) 32:886-90. doi: 10.1179/016164109X12445616596526
55. Vucic S, Burke T, Lenton K, Ramanathan S, Gomes L, Yannikas C, et al. Cortical dysfunction underlies disability in multiple sclerosis. *Mult Scler.* (2012) 18:425-32. doi: 10.1177/1352458511424308

56. Nantes JC, Zhong J, Holmes SA, Narayanan S, Lapierre Y, Koski L. Cortical Damage and Disability in Multiple Sclerosis: Relation to Intracortical Inhibition and Facilitation. *Brain Stimul.* (2016) 9:566-73. doi: 10.1016/j.brs.2016.01.003
57. Nantes JC, Zhong J, Holmes SA, Whatley B, Narayanan S, Lapierre Y, et al. Intracortical inhibition abnormality during the remission phase of multiple sclerosis is related to upper limb dexterity and lesions. *Clin Neurophysiol.* (2016) 127:1503-11. doi: 10.1016/j.clinph.2015.08.011
58. Neva JL, Lakhani B, Brown KE, Wadden KP, Mang CS, Ledwell NH, et al. Multiple measures of corticospinal excitability are associated with clinical features of multiple sclerosis. *Behav Brain Res.* (2016) 297:187-95. doi: 10.1016/j.bbr.2015.10.015
59. Ho KH, Lee M, Nithi K, Palace J, Mills K. Changes in motor evoked potentials to short-interval paired transcranial magnetic stimuli in multiple sclerosis. *Clin Neurophysiol.* (1999) 110:712-9. doi: 10.1016/s1388-2457(98)00048-0
60. Schmierer K, Irlbacher K, Grosse P, Röricht S, Meyer BU. Correlates of disability in multiple sclerosis detected by transcranial magnetic stimulation. *Neurology.* (2002) 59:1218-24. doi: 10.1212/wnl.59.8.1218
61. Lenzi D, Conte A, Mainero C, Frasca V, Fubelli F, Totaro P, et al. Effect of corpus callosum damage on ipsilateral motor activation in patients with multiple sclerosis: a functional and anatomical study. *Hum Brain Mapp.* (2007) 28:636-44. doi: 10.1002/hbm.20305
62. Llufriu S, Blanco Y, Martinez-Heras E, Casanova-Molla J, Gabilondo I, Sepulveda M, et al. Influence of corpus callosum damage on cognition and physical disability in multiple sclerosis: a multimodal study. *PLoS One.* (2012) 7:e37167. doi: 10.1371/journal.pone.0037167
63. Tataroglu C, Genc A, Idiman E, Cakmur R, Idiman F. Cortical silent period and motor evoked potentials in patients with multiple sclerosis. *Clin Neurol Neurosurg.* (2003) 105:105-10. doi: 10.1016/s0303-8467(02)00127-0
64. Hoshiyama M, Kakigi R. Changes of somatosensory evoked potentials during writing with the dominant and non-dominant hands. *Brain Res.* (1999) 833:10-9.
65. Ziemann U. Pharmaco-transcranial magnetic stimulation studies of motor excitability. *Handb Clin Neurol.* (2013) 116:387-97. Review. doi: 10.1016/B978-0-444-53497-2.00032-2
66. Tergau F, Wanschura V, Canelo M, Wischer S, Wassermann EM, Ziemann U, et al. Complete suppression of voluntary motor drive during the silent period after transcranial magnetic stimulation. *Exp Brain Res.* (1999) 124:447–54. doi: 10.1007/s002210050640
67. Santarnecchi E, Rossi S, Bartalini S, Cincotta M, Giovannelli F, Tatti E, et al. Neurophysiological Correlates of Central Fatigue in Healthy Subjects and Multiple Sclerosis Patients before and after Treatment with Amantadine. *Neural Plast.* (2015) 2015:616242. doi: 10.1155/2015/616242
68. Liepert J, Mingers D, Heesen C, Bäumer T, Weiller C. Motor cortex excitability and fatigue in multiple sclerosis: a transcranial magnetic stimulation study. *Mult Scler.* (2005) 11:316-21. doi: 10.1191/1352458505ms1163oa
69. Conte A, Lenzi D, Frasca V, Gilio F, Giacomelli E, Gabriele M, et al. Intracortical excitability in patients with relapsing-remitting and secondary progressive multiple sclerosis. *J Neurol.* (2009) 256:933-8. doi: 10.1007/s00415-009-5047-0
70. Ayache SS, Créange A, Farhat WH, Zouari HG, Lesage C, Palm U, Abdellaoui M, Lefaucheur JP. Cortical excitability changes over time in progressive multiple sclerosis. *Funct Neurol.* (2015) 30:257-63. doi: 10.11138/fneur/2015.30.4.257
71. Schmierer K, Niehaus L, Röricht S, Meyer BU. Conduction deficits of callosal fibres in early multiple sclerosis. *J Neurol Neurosurg Psychiatry.* (2000) 68:633-8. doi: 10.1136/jnnp.68.5.633
72. Jung P, Beyerle A, Humpich M, Neumann-Haefelin T, Lanfermann H, Ziemann U. Ipsilateral silent period: a marker of callosal conduction abnormality in early relapsing-remitting multiple sclerosis? *J Neurol Sci.* (2006) 250:133-9. doi: 10.1016/j.jns.2006.08.008
73. Wahl M, Hübers A, Lauterbach-Soon B, Hattingen E, Jung P, Cohen LG, et al. Motor callosal disconnection in early relapsing-remitting multiple sclerosis. *Hum Brain Mapp.* (2011) 32:846-55. doi: 10.1002/hbm.21071
74. Daskalakis ZJ, Christensen BK, Fitzgerald PB, Roshan L, Chen R. The mechanisms of interhemispheric inhibition in the human motor cortex. *J Physiol.* (2002) 543(Pt 1):317-26.
75. Ayache SS, Chalah MA, Al-Ani T, Farhat WH, Zouari HG, Créange A, et al. Tremor in multiple sclerosis: The intriguing role of the cerebellum. *J Neurol Sci.* (2015) 358:351-6. doi: 10.1016/j.jns.2015.09.360
76. Mori F, Kusayanagi H, Monteleone F, Moscatelli A, Nicoletti CG, Bernardi G, et al. Short interval intracortical facilitation correlates with the degree of disability in multiple sclerosis. *Brain Stimul.* (2013) 6:67-71. doi: 10.1016/j.brs.2012.02.001
77. Ayache SS, Créange A, Farhat WH, Zouari HG, Mylius V, Ahdab R, et al. Relapses in multiple sclerosis: effects of high-dose steroids on cortical excitability. *Eur J Neurol.* (2014) 21:630-6. doi: 10.1111/ene.12356
78. Chaves AR, Wallack EM, Kelly LP, Pretty RW, Wiseman HD, Chen A, et al. Asymmetry of Brain Excitability: A New Biomarker that Predicts Objective and Subjective Symptoms in Multiple Sclerosis. *Behav Brain Res.* (2019) 359:281-91. doi: 10.1016/j.bbr.2018.11.005
79. Induruwa I, Constantinescu CS, Gran B. Fatigue in multiple sclerosis - a brief review. *J Neurol Sci.* (2012). 323:9-15. doi: 10.1016/j.jns.2012.08.007
80. Kos D, Kerckhofs E, Nagels G, D'hooghe MB, Ilsbroukx, S. Origin of Fatigue in Multiple Sclerosis: Review of the Literature. *Neurorehabil Neural Repair*. (2008). 22, 91-100. doi: 10.1177/1545968306298934
81. Vucic S, Burke D, Kiernan MC. Fatigue in multiple sclerosis: mechanisms and management. *Clin Neurophysiol.* (2010) 121:809-17. doi: 10.1016/j.clinph.2009.12.013
82. Chalah MA, Kauv P, Créange A, Hodel J, Lefaucheur JP, Ayache SS. Neurophysiological, radiological and neuropsychological evaluation of fatigue in multiple sclerosis. *Mult Scler Relat Disord*. (2019) 28:145-52. doi: 10.1016/j.msard.2018.12.029
83. Chaves AR, Kelly LP, Moore CS, Stefanelli M, Ploughman M. Prolonged cortical silent period is related to poor fitness and fatigue, but not tumor necrosis factor, in Multiple Sclerosis. *Clin Neurophysiol.* (2019) 130:474-83. doi: 10.1016/j.clinph.2018.12.015
84. Di Filippo M, Portaccio E, Mancini A, Calabresi P. Multiple sclerosis and cognition: synaptic failure and network dysfunction. *Nat Rev Neurosci.* (2018) 19:599-609. doi: 10.1038/s41583-018-0053-9
85. Tokimura H, Di Lazzaro V, Tokimura Y, Oliviero A, Profice P, Insola A, Mazzone P, Tonali P, Rothwell JC. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. *J Physiol.* (2000) 523:503-13.
86. Cucurachi L, Immovilli P, Granella F, Pavesi G, Cattaneo L. Short-latency afferent inhibition predicts verbal memory performance in patients with multiple sclerosis. *J Neurol.* (2008) 255:1949-56. doi: 10.1007/s00415-008-0041-5
87. Di Lazzaro V, Pilato F, Dileone M, Saturno E, Oliviero A, Marra C, Daniele A, Ranieri F, Gainotti G, Tonali PA. In vivo cholinergic circuit evaluation in frontotemporal and Alzheimer dementias. *Neurology.* (2006) 66:1111-3. doi: 10.1212/01.wnl.0000204183.26231.23
88. Chalah MA, Palm U, Lefaucheur JP, Créange A, Ayache SS. Interhermispheric inhibition predicts anxiety levels in multiple sclerosis: A corticospinal excitability study. *Brain Res.* (2018) 1699:186-194. doi: 10.1016/j.brainres.2018.08.029
89. Szczucinski A, Losy J. Chemokines and chemokine receptors in multiple sclerosis. Potential targets for new therapies. *Acta Neurol Scand.* (2007) 115:137-46. https://doi.org/10.1111/j.1600-0404.2006.00749.x
90. Dihb-Jalbut S, Arnold DL, Cleveland DW, Fisher M, Friedlander RM, Mouradian MM. et al. Neurodegeneration and neuroprotection in multiple sclerosis and other neurodegenerative diseases. *J Neuroimmunol.* (2006) 176:198–215. https://doi.org/10.1016/j.jneuroim.2006.03.027.
91. Maimone D, Gregory S, Arnason BG, Reder AT. Cytokine levels in the cerebrospinal fluid and serum of patients with multiple sclerosis. *J Neuroimmunol.* (1991) 32:67–74. https://doi.org/10.1016/0165-5728(91)90073-G
92. Matejčíková Z, Mareš J, Sládková V, Svrčinová T, VysloužilováJ, Zapletalová J. et al. Cerebrospinal fluid and serum levels of interleukin-8 in patients with multiple sclerosis and its correlation with Q-albumin. *Mult Scler Relat Disord.* (2017) 14:12–5. doi: 10.1016/j.msard .2017.03.007
93. Kothur K, Wienholt L, Brilot F, Dale RC. CSF cytokines/chemokines as biomarkers in neuroinflammatory CNS disorders: a systematic review. *Cytokine.* (2016) 7:227–237. doi: 10.1016/j.cyto.2015.10.001

94. Göbel K, Ruck T, Meuth SG. Cytokine signaling in multiple sclerosis: Lost in translation. *Mult Scler J.* (2018) 24:432–439. https://doi.org/10.1177/1352458518763094
95. Cannella B, Raine CS. Multiple sclerosis: cytokine receptors on oligodendrocytes predict innate regulation. *Ann Neurol.* (2004) J55:46-57. doi: 10.1002/ana.10764
96. Werneburg S, Feinberg PA, Johnson KM, Schafer DP. A microglia-cytokine axis to modulate synaptic connectivity and function. *Curr Opin Neurobiol.* (2017) 47:138-45. doi: 10.1016/j.conb.2017.10.002
97. Vezzani A, Balosso S, Ravizza T. The role of cytokines in the pathophysiology of epilepsy. *Brain Behav Immun.* (2008) 22:797-803. doi: 10.1016/j.bbi.2008.03.009
98. Vezzani A, Viviani B. Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability. *Neuropharmacology.* (2015) 96:70-82. doi: 10.1016/j.neuropharm.2014.10.027
99. Wu Y, Dissing-Olesen L, MacVicar BA, Stevens B. Microglia: Dynamic Mediators of Synapse Development and Plasticity. *Trends Immunol.* (2015) 36:605-613. doi: 10.1016/j.it.2015.08.008
100. Tian L, Ma L, Kaarela T, Li Z. Neuroimmune crosstalk in the central nervous system and its significance for neurological diseases. *J Neuroinflammation.* (2012) 9:155. doi: 10.1186/1742-2094-9-155
101. Rizzo FR, Musella A, De Vito F, Fresegna D, Bullitta S, Vanni V, et al. Tumor Necrosis Factor and Interleukin-1β Modulate Synaptic Plasticity during Neuroinflammation. *Neural Plast.* (2018) 2018:8430123. doi: 10.1155/2018/8430123
102. Mandolesi G, Musella A, Gentile A, Grasselli G, Haji N, Sepman H, et al. Interleukin-1β alters glutamate transmission at purkinje cell synapses in a mouse model of multiple sclerosis. *J Neurosci.* (2013) 33:12105-21. doi: 10.1523/JNEUROSCI.5369-12.2013
103. Takayasu Y, Iino M, Ozawa S. Roles of glutamate transporters in shaping excitatory synaptic currents in cerebellar Purkinje cells. *Eur J Neurosci.* (2004) 19:1285–95. doi: 10.1111/j.1460-9568.2004.03224.x
104. Seckinger P, Lowenthal JW, Williamson K, Dayer JM, MacDonald HR. A urine inhibitor of interleukin 1 activity that blocks ligand binding. *J Immunol.* (1987) 139:1546–9.
105. Furlan R, Bergami A, Brambilla E, Butti E, De Simoni MG, Campagnoli M, et al. HSV-1-mediated IL-1 receptor antagonist gene therapy ameliorates MOG(35–55)-induced experimental autoimmune encephalomyelitis in C57BL/6 mice. Gene Ther (2007) 14:93–8. doi: 10.1038/sj.gt.3302805
106. Rossi S, Muzio L, De Chiara V, Grasselli G, Musella A, Musumeci G, et al. Impaired striatal GABA transmission in experimental autoimmune encephalomyelitis. *Brain Behav Immun.* (2011) 25:947-56. doi: 10.1016/j.bbi.2010.10.004
107. Mandolesi G, Grasselli G, Musella A, Gentile A, Musumeci G, Sepman H, et al. GABAergic signaling and connectivity on Purkinje cells are impaired in experimental autoimmune encephalomyelitis. *Neurobiol Dis.* (2012) 46:414-24. doi: 10.1016/j.nbd.2012.02.005
108. Bhat R, Axtell R, Mitra A, Miranda M, Lock C, Tsien RW, et al. Inhibitory role for GABA in autoimmune inflammation. *Proc Natl Acad Sci USA*. (2010) 107:2580–5. doi: 10.1073/pnas.0915139107
109. Rossi S, Furlan R, De Chiara V, Motta C, Studer V, Mori F, et al. Interleukin-1β causes synaptic hyperexcitability in multiple sclerosis. *Ann Neurol.* (2012) 71:76-83. doi: 10.1002/ana.22512
110. Gentile A, De Vito F, Fresegna D, Rizzo FR, Bullitta S, Guadalupi L, et al. Peripheral T cells from multiple sclerosis patients trigger synaptotoxic alterations in central neurons. *Neuropathol Appl Neurobiol.* (2019) [Epub ahead of print] doi: 10.1111/nan.12569
111. Caramia MD, Palmieri MG, Desiato MT, Boffa L, Galizia P, Rossini PM, et al. Brain excitability changes in the relapsing and remitting phases of multiple sclerosis: a study with transcranial magnetic stimulation. *Clin Neurophysiol.* (2004) 115:956-65. doi: 10.1016/j.clinph.2003.11.024
112. Rossi S, Studer V, Motta C, Germani G, Macchiarulo G, Buttari F, et al. Cerebrospinal fluid detection of interleukin-1β in phase of remission predicts disease progression in multiple sclerosis. *J Neuroinflammation.* (2014) 11:32. doi: 10.1186/1742-2094-11-32
113. Pittaluga A. CCL5-Glutamate Cross-Talk in Astrocyte-Neuron Communication in Multiple Sclerosis. *Front Immunol.* (2017) 8:1079. doi: 10.3389/fimmu.2017.01079
114. Mori F, Nisticò R, Nicoletti CG, Zagaglia S, Mandolesi G, Piccinin S, et al. RANTES correlates with inflammatory activity and synaptic excitability in multiple sclerosis. *Mult Scler.* (2016) 22:1405-12. doi: 10.1177/1352458515621796
115. Rossi S, Motta C, Studer V, Barbieri F, Buttari F, Bergami A, Sancesario G, Bernardini S, De Angelis G, Martino G, Furlan R, Centonze D. Tumor necrosis factor is elevated in progressive multiple sclerosis and causes excitotoxic neurodegeneration. *Mult Scler.* (2014) 20:304-12. doi: 10.1177/1352458513498128
116. Zhou Z, Peng X, Insolera R, Fink DJ, Mata M. IL-10 promotes neuronal survival following spinal cord injury. *Exp Neurol.* (2009) 220:183-90. doi: 10.1016/j.expneurol.2009.08.018
117. Lobo-Silva D, Carriche GM, Castro AG, Roque S, Saraiva M. Balancing the immune response in the brain: IL-10 and its regulation. *J Neuroinflammation.* (2016) 13:297. doi: 10.1186/s12974-016-0763-8
118. Tseng HC, Dichter MA. Platelet-derived growth factor-BB pretreatment attenuates excitotoxic death in cultured hippocampal neurons. *Neurobiol Dis.* (2005) 19:77–83. doi: 10.1016/j.nbd.2004.11.007
119. Cheng B, Mattson MP. PDGFs protect hippocampal neurons against energy deprivation and oxidative injury: evidence for induction of antioxidant pathways. *J Neurosci.* (1995) 15:7095-104.
120. Rossi S, Mancino R, Bergami A, Mori F, Castelli M, De Chiara V, et al. Potential role of IL-13 in neuroprotection and cortical excitability regulation in multiple sclerosis. *Mult Scler.* (2011) 17:1301-12. doi: 10.1177/1352458511410342

Conflict of Interest: The authors declare the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: FB acted as Advisory Board members of 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. DC 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, or in the decision to publish the results.

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

frontiers in Neurology

MINI REVIEW
published: 15 July 2020
doi: 10.3389/fneur.2020.00552

Can Operant Conditioning of EMG-Evoked Responses Help to Target Corticospinal Plasticity for Improving Motor Function in People With Multiple Sclerosis?

Aiko K. Thompson[1]* *and Thomas Sinkjær*[2,3]

[1] Department of Health Sciences and Research, College of Health Professions, Medical University of South Carolina, Charleston, SC, United States, [2] Department of Health Science and Technology, Aalborg University, Aalborg, Denmark, [3] Lundbeck Foundation, Copenhagen, Denmark

OPEN ACCESS

Edited by:
Moussa Antoine Chalah, Hôpitaux Universitaires Henri Mondor, France

Reviewed by:
James W. Grau, Texas A&M University, United States
Tomofumi Yamaguchi, Yamagata Prefectural University of Health Sciences, Japan
Vincenzo Di Lazzaro, Campus Bio-Medico University, Italy

***Correspondence:**
Aiko K. Thompson
thompsai@musc.edu

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: 31 January 2020
Accepted: 15 May 2020
Published: 15 July 2020

Citation:
Thompson AK and Sinkjær T (2020) Can Operant Conditioning of EMG-Evoked Responses Help to Target Corticospinal Plasticity for Improving Motor Function in People With Multiple Sclerosis? Front. Neurol. 11:552. doi: 10.3389/fneur.2020.00552

Corticospinal pathway and its function are essential in motor control and motor rehabilitation. Multiple sclerosis (MS) causes damage to the brain and descending connections, and often diminishes corticospinal function. In people with MS, neural plasticity is available, although it does not necessarily remain stable over the course of disease progress. Thus, inducing plasticity to the corticospinal pathway so as to improve its function may lead to motor control improvements, which impact one's mobility, health, and wellness. In order to harness plasticity in people with MS, over the past two decades, non-invasive brain stimulation techniques have been examined for addressing common symptoms, such as cognitive deficits, fatigue, and spasticity. While these methods appear promising, when it comes to motor rehabilitation, just inducing plasticity or having a capacity for it does not guarantee generation of better motor functions. Targeting plasticity to a key pathway, such as the corticospinal pathway, could change what limits one's motor control and improve function. One of such neural training methods is operant conditioning of the motor-evoked potential that aims to train the behavior of the corticospinal-motoneuron pathway. Through up-conditioning training, the person learns to produce the rewarded neuronal behavior/state of increased corticospinal excitability, and through iterative training, the rewarded behavior/state becomes one's habitual, daily motor behavior. This minireview introduces operant conditioning approach for people with MS. Guiding beneficial CNS plasticity on top of continuous disease progress may help to prolong the duration of maintained motor function and quality of life in people living with MS.

Keywords: operant conditioning, motor-evoked potential, corticospinal excitability, foot drop, plasticity

INTRODUCTION

Over the past 15 years, the awareness of the importance of physical rehabilitation and exercise has been steadily growing in the field of multiple sclerosis (MS)-related research (1–3). This trend should continue, with ongoing development and testing of disease-modifying drugs (4), which will lead to prolonging disease stability and creating greater opportunities for reducing motor

impairments, improving mobility, and improving quality of life in people with MS, as pointed out by Ploughman (3). Although underlying mechanisms may not be fully understood (5), mounting evidence indicates positive effects of exercise on physical fitness, balance and mobility, cognitive function, participation, and other outcomes (1, 6). A challenge is that a person with MS may not be able to appreciate the greatness of exercise, when reduced movement efficiency and impaired mobility make it difficult for him/her to be engaged in physical activity. Without changing what is available to execute essential daily motor function such as gait, and without changing what is limiting one's function, movement dysfunction would continue to limit mobility and quality of life in people with MS. While disease progress continuously alters one's physiology, it is essential to guide the central nervous system (CNS) plasticity that can help to prolong the duration of maintained motor function and quality of life in people living with MS.

In this brief review, we will discuss the corticospinal plasticity in people with MS and introduce operant conditioning approach as a method to target plasticity in the corticospinal pathway for improving motor function in people with MS.

CNS PLASTICITY IN PEOPLE WITH MS

MS is a chronic inflammatory, autoimmune disease of the CNS. In persons with MS, neurological deficits are commonly attributed to inflammatory demyelination in the CNS and damage to the gray matter in cortical and subcortical structures, with lesion patterns, locations, volumes, and their rates of changes differing among subtypes of MS (7–10). In addition to accumulating structural damage, the process of inflammation itself affects synaptic transmission and plasticity (11). Elevation in the level of inflammatory cytokines not only changes glutamatergic and GABAergic transmissions, which lead to synaptic hyperexcitability and excitotoxicity, but also affects synaptic plasticity (11–14), which is essential for clinical and functional recovery. Thus, from damage to the brain and the descending pathways and from alteration in synaptic plasticity, disruption of corticospinal function is a hard-to-avoid problem in people with MS (15–17).

Transcranial magnetic stimulation (TMS), its motor-evoked potential (MEP), and their associated measures, such as short- and long-interval cortical inhibition (SICI and LICI), short-interval cortical facilitation (SICF), and intracortical facilitation (ICF), are useful tools for investigating cortical and corticospinal plasticity (18–22). They are also useful in detecting and predicting the progression of disability and recovery (15, 16, 23–27). For instance, small MEPs with long latencies, high motor thresholds, and prolonged cortical silent periods tend to correlate with the Expanded Disability Status Scale (EDSS) scores (15–17, 28–30). Silent period (SP) after MEP, known to reflect cortical inhibition at least partly (31–37), is reduced in the relapsing or progressive phases of MS (38, 39), whereas the SP is prolonged in the remitting phase (38). In the stable phase of relapsing–remitting MS individuals, SICI and ICF could be similar to those of the control group (17). A common observation is that cortical inhibition is reduced during the relapsing or progressive phase, whereas the inhibition is clearly present during the stable or remitting phase (11); the phase or state of disease appears to be reflected in the measured cortical inhibition. In addition, how these measures respond to plasticity-inducing neuromodulation can suggest the availability of plasticity at the time of assessment and help to predict recovery from relapse (11–13, 23, 40).

The availability of synaptic plasticity, also known as "plasticity reserve" (11), can be measured in persons with MS by applying plasticity-inducing neuromodulation techniques, such as repetitive TMS (rTMS) at high (e.g., 20 Hz) or low (e.g., 1 Hz) frequency, rTMS with intermittent or continuous theta burst stimulation patterns (iTBS and cTBS) (11, 13, 14, 41, 42), paired associative stimulation (PAS) with TMS, and peripheral nerve stimulation (PNS) (12, 23, 43, 44). They can be used to assess long-term potentiation (with high-frequency rTMS and iTBS), long-term depression (with low-frequency rTMS and cTBS), and spike-timing-dependent Hebbian-type plasticity (with PAS) (11, 13, 42). For example, in individuals with primary progressive MS, neither iTBS nor cTBS exert the expected plasticity effects; in individuals in the relapsing phase of MS, iTBS produces expected LTP effects, but cTBS fails to produce expected LTD effects (42). This plasticity reserve may be an essential mechanism of clinical symptom and disability progression in MS; when plasticity reserve is exhausted and synaptic plasticity is unavailable, surviving neurons would not be able to compensate for neuronal loss (11).

Importantly, while people with MS can display plasticity (43, 45–48), there is no guarantee that their plasticity adaptive to progressive neuronal damage is beneficial; it may exaggerate or lessen clinical symptoms (42). Thus, to guide the plasticity in beneficial directions, a neurobehavioral training should be incorporated into MS rehabilitation. For improving impaired motor function in people with MS, it would be critically important to induce and maintain beneficial plasticity in the corticospinal pathway, as its function is the foundation of voluntary and involuntary motor behaviors.

NEUROMODULATION FOR REHABILITATION IN PEOPLE WITH MS

There are a wide variety of neurorehabilitation interventions currently available or being tested for individuals with CNS disorders, including MS (https://clinicaltrials.gov). Many of those expect to induce cortical and/or subcortical plasticity and may improve sensorimotor function [e.g., (49–52)]. Of different neuromodulation approaches, there have been growing interests in non-invasive brain stimulation (NIBS); in particular, rTMS and transcranial direct current stimulation (tDCS) have been increasingly utilized for treating various MS symptoms (41, 53–59). Other neuromodulation methods, such as deep brain stimulation and spinal cord stimulation, have been reviewed in (60). As effects and mechanisms of rTMS and tDCS have been thoroughly covered in recent reviews (54–56, 58), these methods will not be further discussed in this minireview. However, it is worth reiterating that studies of LTP or LTD-inducing

rTMS (e.g., iTBS, low-frequency rTMS) and tDCS that affects polarization of the stimulated cortical network have shown some promising results; common MS symptoms, such as fatigue, cognitive functions, pain, and spasticity, can be alleviated by these methods (40, 41, 53, 54, 56–58, 61, 62).

When applying NIBS for improving impaired motor function, consideration on how to guide the stimulation-induced plasticity is critically important. Because NIBS-induced plasticity is rather widespread and not pathway specific, without an additional strategy to shape such plasticity into functionally beneficial changes, many changes at many different sites could compensate for each other, toward maintaining the state of neural network at net change of zero [i.e., homeostatic plasticity (63–69)]. Thus, pairing two interventions, e.g., iTBS + exercise (70), could be a logical NIBS application strategy for motor rehabilitation. Task-specific PNS, such as FES for foot drop (71–73), with which functional movement and phase-specific PNS occur concurrently, uniquely emulates a neuromodulation combination strategy, increases MEP amplitude, and improves motor function in people with MS and other neuromuscular disorders (49, 74).

Another class of neuromodulation methods include PAS (23, 43, 75–79) and operant conditioning of muscle [electromyographic (EMG)]-evoked potentials (80–82), which target plasticity in a specific pathway. Detailed mechanisms of PAS approaches have been discussed in (75, 76, 78, 83, 84). Briefly, with PAS protocols that induces spike-timing-dependent plasticity (76–78), synaptic transmission can be potentiated or depressed depending on the relative timing between the presynaptic and postsynaptic spiking (77, 85, 86), and repeated application of TMS-PNS PAS can potentiate corticospinal-motoneuronal transmission and excitability in people with MS (43, 45). A similar PAS concept can also be applied to cortical neurons (79, 87, 88). Therapeutic potency of PAS in people with MS is yet to be determined.

OPERANT CONDITIONING OF EMG-EVOKED POTENTIALS

Operant conditioning is a method for modifying a behavior based on the consequence of that behavior (89). Usually, when a person acquires a new behavior through operant conditioning, s/he does not need to discover the operant contingency through trial and error. However, when this approach is applied to a behavior of a neural pathway (e.g., a reflex), an individual must go through a trial-and-error discovery phase, as s/he would not have prior knowledge on how to control volitionally a behavior or the excitability of that specific pathway. Thus, with operant conditioning of an EMG-evoked potential that reflects the behavior and/or excitability of a certain neural pathway, a subject learns to produce a neuronal behavior that is rewarded through trial and error, similarly between humans and animals (89). Through repetition, the rewarded behavior can become a habitual behavior (90). With operant conditioning of an EMG-evoked potential, such as a reflex and an MEP, a subject is rewarded only for increasing or decreasing a target pathway's excitability (81, 82). Thus, over time, it changes the pathway that produces that response (82).

By changing the transmission of a key pathway with a directional aim (up/down), operant conditioning of an EMG-evoked potential seeks to improve the targeted pathway's function and enable more effective movements in which the targeted pathway contributes (91, 92). An emerging theory is that changing a key pathway leads to a cascade of wider beneficial changes in the activity of other spinal and supraspinal pathways (81, 93), impacting motor function recovery.

Much of the physiological and theoretical knowledge of operant conditioning approach is based on a large number of reflex conditioning studies (68, 82, 94). The most essential includes the following. An operantly conditioned reflex behavior rests on a hierarchy of plasticity from the brain to the spinal cord (68, 82, 95, 96). The reward contingency produces plasticity in the brain that induces and maintains the spinal cord plasticity that is directly responsible for the conditioned reflex behavior (68, 82, 94). Among the major descending pathways, the corticospinal tract is the only pathway essential for conditioning-induced plasticity (97). Thus, when the corticospinal tract and its plasticity are preserved at least partially, the targeted change can be induced through conditioning (98), which then changes how that reflex pathway functions in complex motion such as locomotion (80, 92, 99). These provide the foundation for currently emerging clinical applications of MEP operant conditioning.

OPERANT CONDITIONING OF THE MOTOR-EVOKED POTENTIALS

As in reflex operant conditioning (68, 81, 82, 100–102), operantly up-conditioning the MEP can increase the corticospinal excitability for the targeted muscle in people (91, 103). In the first 100–1,000 up-conditioning trials, a person learns through trial and error how to increase MEP size, and MEP size gradually increases over the subsequent conditioning sessions (**Figure 1**). Motivation is critical in operant conditioning (89, 90, 106, 107); the person must value the positive feedback that s/he receives from producing a larger MEP. Our studies suggest that in individuals with CNS disorders, who take the conditioning trials more seriously than those without CNS injury, MEP size is highly likely to increase, and their MEP increase can persist at least a few months after conditioning ends (103, 104).

Two key factors underline the therapeutic potency of MEP conditioning. First, it targets plasticity to the corticospinal pathway that produces an MEP in the targeted muscle. The protocol prohibits change in the background EMG activity; the individual is rewarded only for increasing the target muscle's MEP (i.e., for increasing corticospinal excitability for the target muscle). This pathway specificity differentiates MEP conditioning from EMG biofeedback training (108–112) or muscle strength training (113–119), both of which are not tailored for modulating or controlling the excitability or behavior a specific pathway. While practice is essential in improving motor performance, movement practice alone could let an individual easily default to relying on what is readily

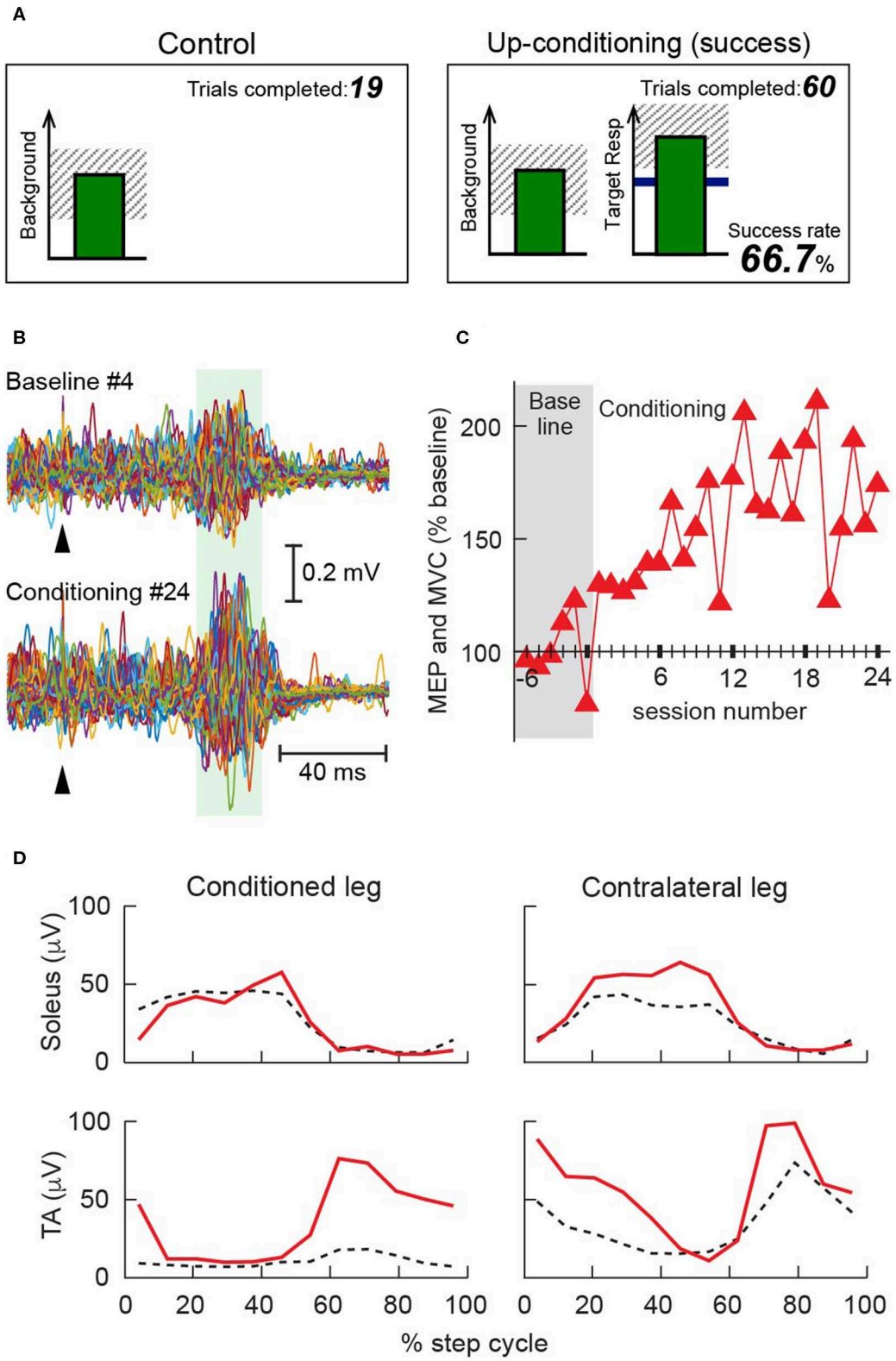


FIGURE 1 | Operant conditioning of the tibialis anterior (TA) motor-evoked potential (MEP) in individuals with multiple sclerosis (MS) [modified from (104)]. **(A)** Visual feedback screens for MEP control and MEP operant conditioning trials. In all trials, the number of the current trial within its block is displayed, and the background

(Continued)

FIGURE 1 | electromyographic (EMG) panel shows the correct range (shaded) and the current value (green vertical bar, updated every 200 ms). If TA EMG activity stays in the correct range for at least 2 s and at least 5.5 s has passed since the last trial, an MEP is elicited. In control trials (left), the MEP panel is not shown. In conditioning trials (right), the shading in the MEP panel indicates the rewarded MEP range for up-conditioning. The dark horizontal line is the average MEP size of the baseline sessions, and the vertical bar is the MEP size, calculated in the MEP interval of that specific individual [e.g., 45–70 ms after transcranial magnetic stimulation (TMS)], for the most recent trial. The vertical bar appears 200 ms after TMS. If that MEP size reaches into the shaded area, the bar is green, and the trial is a success. If it falls below the shaded area, the bar is red, and the trial is a not a success. The running success rate for the current block is shown at the bottom. **(B)** Examples of TA MEP in a 56-year-old woman with MS (Expanded Disability Status Scale 4.0* at baseline). Peristimulus EMG sweeps from the fourth baseline session (top) and the 24th conditioning session (bottom). For each part, 75 sweeps are superimposed. A green shaded band indicates the time window for her MEP size calculation. Arrowheads indicate the time of TMS. **(C)** Mean MEP size (i.e., the mean of 225 control MEP trials in baseline sessions or 225 conditioned MEP trials in conditioning sessions) in 6 baseline and 24 conditioning sessions that occurred at a rate of 3 sessions/week. Over the course of conditioning, her MEP size increased progressively; the final MEP size was 175% of the baseline value. **(D)** Rectified locomotor EMG activity in soleus and TA bilaterally before (dashed black) and after (solid red) conditioning. The step cycle, from foot contact to the end of swing phase, is divided into 12 equal bins. After TA MEP up-conditioning, swing phase TA burst increased in the conditioned leg, which helped this individual regain ankle dorsiflexion and eliminated foot drop. The swing phase burst was also increased in the contralateral TA. All panels have been adapted from (104) with permission. *EDSS 4.0 (105): Fully ambulatory without aid, self-sufficient, up and about some 12 h a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combination of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 500 m.

available (e.g., trying to rely on the hip flexors, instead of improving corticospinal drive to the impaired ankle dorsiflexors), leaving a key pathway unchanged. Because MEP up-conditioning increases the excitability of the corticospinal pathway for the target muscle, it affects motor skills, such as locomotion, to which the pathway contributes. Thus, with the ankle dorsiflexor tibialis anterior (TA) MEP up-conditioning (**Figure 1**), locomotion can be improved in people suffering from foot drop (weak ankle dorsiflexion) (91, 104).

Second, by improving the function of a key pathway, corticospinal pathway, MEP conditioning can trigger further beneficial changes in the activity of other CNS pathways (80, 81, 93, 120), changing what is possible/available in one's recovery path. By targeting the weakened corticospinal drive to the TA and ameliorating the locomotor impediment of foot drop, TA MEP conditioning can enable more effective execution of locomotion; this would then induce wider beneficial plasticity. Increased corticospinal drive to the conditioned TA (49) can explain increases in TA MEP and TA burst amplitude during the swing phase of locomotion observed in people with MS and SCI (91, 104) but cannot explain widespread bilateral improvements in locomotor EMG activity (91, 104) (**Figure 1**). These wider effects of MEP conditioning are similar to those of the soleus H-reflex down-conditioning, with which proximal and distal leg muscles' locomotor EMG improved bilaterally in people with SCI (92). How an operant-conditioning acquired new skill of changing a specific pathway's excitability would trigger a widespread adaptive plasticity in many spinal/supraspinal pathways has been addressed in a theory of system function known as the negotiated equilibrium model (68, 93).

EFFECTS OF MEP CONDITIONING AND CORTICOSPINAL PLASTICITY IN PEOPLE WITH MS

Among people with foot drop due to MS or SCI, locomotor TA activity improved and walking speed increased while MEP increased (91, 103, 104). Conditioning-induced MEP increase was often accompanied by systematic decrease in SP duration (103, 104). SP is known to reflect cortical inhibition at least partly (31–37), and different neural circuits underlie MEP and SP (121–123). If MEP up-conditioning simply increased the general excitability of the cortex, both MEP and SP would have increased [e.g., (124)]. This was not the case. Instead, there were some selective effects on excitatory and inhibitory neurons in the cortex (125, 126). Since reduction in intracortical inhibition occurs through modulation of GABAergic inhibitory interneurons (127–131), it is highly likely that GABAergic inhibitory mechanisms are involved in conditioning-induced SP changes. SPs are often prolonged in people with stable or secondary progressive MS (28, 29, 132), which likely reflects altered $GABA_B$-mediated intracortical inhibition (33, 131, 133, 134). Despite an altered state of cortical inhibition in preconditioning, MEP up-conditioning could reduce SP in individuals with stable MS (104). Further investigation is clearly needed to understand the mechanisms and effects of MEP up-conditioning on cortical inhibition in MS.

OPERANT CONDITIONING OF SPINAL REFLEXES

In addition to MEP conditioning protocols, several reflex conditioning protocols are currently being developed. To date, two protocols have been systematically tested in people with or without CNS damage: the soleus short-latency stretch reflex (known as M1 response) conditioning, using mechanical joint perturbation (135), and the soleus H-reflex conditioning, which uses electrical stimulation of the tibial nerve (92, 99, 102, 136). With both stretch and H-reflex conditioning protocols, the person learns to increase or decrease the target reflex size over 24–30 conditioning sessions. The protocols are designed to induce sustaining changes in descending influence over the reflex pathway, which in turn, produce targeted plasticity in that pathway (101). Because these protocols can change the transmission of targeted pathways, they can be designed to address the specific functional deficits of an individual. For example, in people with spastic hyperreflexia due to incomplete SCI, down-conditioning of the soleus H-reflex pathway, whose hyperactivity impaired locomotion, could improve their locomotion (80, 92). Down-conditioning of the

stretch or H-reflex might also improve spasticity and spastic movement disorders in people with MS (137). It should also be possible to condition other important pathways, such as pathways of spinal reciprocal and presynaptic inhibition (138–140), for further improving their motor functions.

OPERANT CONDITIONING IN MS: CHALLENGES AND POSSIBILITIES

Up until now, the majority of evoked potential operant conditioning studies have been done in SCI (68, 80, 82, 92, 99, 141–145), and its investigation in MS is still in an early stage. Unique challenges in the MS population that do not necessarily apply to the SCI population include impaired cognitive function, fatigue, and ongoing and/or recurring inflammation (14, 56, 59, 146). Since operant conditioning is a behavioral learning approach (81, 82, 89), impairments in learning, memory, and attention that are frequently found in MS may affect the effectiveness of this approach in people with MS. The fact that recurring inflammation influences synaptic plasticity and plasticity reserve (11, 13), which are physiological mechanism of learning, memory, and function recovery, could well interfere with induction and maintenance of conditioning-induced beneficial plasticity. Furthermore, extents of these challenges could vary among MS subtypes and across different individuals (11, 17, 38, 42). Clearly, more studies are needed to determine the applicability of operant conditioning approach in people with MS, and an investigation needs to include persons with all MS subtypes. Long-term follow-up should also be part of such investigations, although often unpredictable disease progress may mask or reduce the induction of plasticity and function improvements temporally or permanently (23, 44, 49, 74). Over 3.5 years of follow-up with a woman with secondary progressive MS supports a possibility of long-term maintenance of corticospinal transmission and function improvements with MEP operant conditioning (104).

A possible strategy to overcome the above-mentioned MS-related challenges is coadministration of conditioning training with NIBS or pharmacological treatment. Reflex or MEP conditioning that aims to change behaviors of the targeted pathway is fundamentally different from rTMS and tDCS, or pharmacological treatments, such as baclofen (147, 148). Because the mechanisms of action differ so vastly from each other, with careful consideration of dosing schedules and individual or combined effects, it may be possible to enhance functional outcomes by coadministering a conditioning protocol with another intervention. Drugs such as dalfampridine and D-aspartate (149–153) may further enhance the corticospinal plasticity and transmission improvement produced by MEP conditioning.

CONCLUSION

A growing number of neurophysiological studies indicate the importance of neuroplasticity and its management for neurorehabilitation in people with MS (11, 13, 54, 56–60, 154). While the benefit of exercise in health and wellness has become recognized (1, 6), investigation on how to improve impaired motor function and mobility, which can limit one's ability to exercise, has been left behind (3). Applying neural training methods, such as operant conditioning of EMG-evoked potentials, to guide beneficial plasticity in the corticospinal or other important CNS pathways may minimize the factors that limit function improvement in people with MS. As CNS plasticity remains available over many years of disease progress (43, 46, 47), guiding it appropriately to gain function improvements on top of changing physiology may help to prolong the duration of maintained motor function and quality of life in people with MS.

AUTHOR CONTRIBUTIONS

AT drafted, edited, and revised the manuscript. TS edited and revised the manuscript. Both authors read and approved the final version submitted for publication.

FUNDING

This work was supported in part by Sawmill owner Jeppe Juhl's and wife Ovita Juhl's Memorial Legate and Spar Nord Fonden of Denmark, the US National Institute of Neurological Disorders and Stroke (R01NS114279), the US National Institute of Biomedical Imaging and Bioengineering (P41EB018783), the US National Institute of General Medical Sciences [GM104941, Institutional Development Award (IDeA) to Binder-MacLeod], and the Doscher Neurorehabilitation Research Program.

REFERENCES

1. Motl RW, Sandroff BM, Kwakkel G, Dalgas U, Feinstein A, Heesen C, et al. Exercise in patients with multiple sclerosis. *Lancet Neurol.* (2017) 16:848–56. doi: 10.1016/S1474-4422(17)30281-8
2. Motl RW, Goldman MD, Benedict RH. Walking impairment in patients with multiple sclerosis: exercise training as a treatment option. *Neuropsychiatr Dis Treat.* (2010) 6:767–74. doi: 10.2147/NDT.S10480
3. Ploughman M. A new era of multiple sclerosis rehabilitation: lessons from stroke. *Lancet Neurol.* (2017) 16:768–9. doi: 10.1016/S1474-4422(17)30301-0
4. Gerardi C, Bertele V, Rossi S, Garattini S, Banzi R. Preapproval and postapproval evidence on drugs for multiple sclerosis. *Neurology.* (2018) 90:964–73. doi: 10.1212/WNL.0000000000005561
5. Negaresh R, Motl RW, Mokhtarzade M, Dalgas U, Patel D, Shamsi MM, et al. Effects of exercise training on cytokines and adipokines in multiple sclerosis: a systematic review. *Mult Scler Relat Disord.* (2018) 24:91–100. doi: 10.1016/j.msard.2018.06.008
6. Motl RW, Pilutti LA. The benefits of exercise training in multiple sclerosis. *Nat Rev Neurol.* (2012) 8:487–97. doi: 10.1038/nrneurol.2012.136
7. Klaver R, de Vries HE, Schenk GJ, Geurts JJ. Grey matter damage in multiple sclerosis: a pathology perspective. *Prion.* (2013) 7:66–75. doi: 10.4161/pri.23499
8. Nijeholt GJ, van Walderveen MA, Castelijns JA, van Waesberghe JH, Polman C, Scheltens P, et al. Brain and spinal cord abnormalities in multiple sclerosis. Correlation between MRI parameters, clinical subtypes and symptoms. *Brain.* (1998) 121(Pt 4):687–97. doi: 10.1093/brain/121.4.687

9. Calabrese M, Magliozzi R, Ciccarelli O, Geurts JJ, Reynolds R, Martin R. Exploring the origins of grey matter damage in multiple sclerosis. *Nat Rev Neurosci.* (2015) 16:147–58. doi: 10.1038/nrn3900
10. Datta G, Colasanti A, Rabiner EA, Gunn RN, Malik O, Ciccarelli O, et al. Neuroinflammation and its relationship to changes in brain volume and white matter lesions in multiple sclerosis. *Brain.* (2017) 140:2927–38. doi: 10.1093/brain/awx228
11. Stampanoni Bassi M, Mori F, Buttari F, Marfia GA, Sancesario A, Centonze D, et al. Neurophysiology of synaptic functioning in multiple sclerosis. *Clin Neurophysiol.* (2017) 128:1148–57. doi: 10.1016/j.clinph.2017.04.006
12. Stampanoni Bassi M, Iezzi E, Mori F, Simonelli I, Gilio L, Buttari F, et al. Interleukin-6 disrupts synaptic plasticity and impairs tissue damage compensation in multiple sclerosis. *Neurorehabil Neural Repair.* (2019) 33:825–35. doi: 10.1177/1545968319868713
13. Nistico R, Mori F, Feligioni M, Nicoletti F, Centonze D. Synaptic plasticity in multiple sclerosis and in experimental autoimmune encephalomyelitis. *Philos Trans R Soc Lond B Biol Sci.* (2014) 369:20130162. doi: 10.1098/rstb.2013.0162
14. Mori F, Rossi S, Sancesario G, Codeca C, Mataluni G, Monteleone F, et al. Cognitive and cortical plasticity deficits correlate with altered amyloid-beta CSF levels in multiple sclerosis. *Neuropsychopharmacology.* (2011) 36:559–68. doi: 10.1038/npp.2010.187
15. Kale N, Agaoglu J, Onder G, Tanik O. Correlation between disability and transcranial magnetic stimulation abnormalities in patients with multiple sclerosis. *J Clin Neurosci.* (2009) 16:1439–42. doi: 10.1016/j.jocn.2009.03.009
16. Gagliardo A, Galli F, Grippo A, Amantini A, Martinelli C, Amato MP, et al. Motor evoked potentials in multiple sclerosis patients without walking limitation: amplitude vs conduction time abnormalities. *J Neurol.* (2007) 254:220–7. doi: 10.1007/s00415-006-0334-5
17. Conte A, Lenzi D, Frasca V, Gilio F, Giacomelli E, Gabriele M, et al. Intracortical excitability in patients with relapsing-remitting and secondary progressive multiple sclerosis. *J Neurol.* (2009) 256:933–8. doi: 10.1007/s00415-009-5047-0
18. Hallett M. Transcranial magnetic stimulation: a primer. *Neuron.* (2007) 55:187–99. doi: 10.1016/j.neuron.2007.06.026
19. Pascual-Leone A, Davey NJ, Rothwell J, Wassermann EM, Puri BK. *Handbook of Transcranial Magnetic Stimulation*. London: Arnold, a member of the Hodder Headline Group (2002).
20. Pascual-Leone A, Walsh V, Rothwell J. Transcranial magnetic stimulation in cognitive neuroscience–virtual lesion, chronometry, functional connectivity. *Curr Opin Neurobiol.* (2000) 10:232–7. doi: 10.1016/S0959-4388(00)00081-7
21. Hallett M. Transcranial magnetic stimulation and the human brain. *Nature.* (2000) 406:147–50. doi: 10.1038/35018000
22. Cohen LG, Ziemann U, Chen R, Classen J, Hallett M, Gerloff C, et al. Studies of neuroplasticity with transcranial magnetic stimulation. *J Clin Neurophysiol.* (1998) 15:305–24. doi: 10.1097/00004691-199807000-00003
23. Mori F, Kusayanagi H, Nicoletti CG, Weiss S, Marciani MG, Centonze D. Cortical plasticity predicts recovery from relapse in multiple sclerosis. *Mult Scler.* (2013) 20:451–7. doi: 10.1177/1352458513512541
24. Schlaeger R, D'Souza M, Schindler C, Grize L, Dellas S, Radue EW, et al. Prediction of long-term disability in multiple sclerosis. *Mult Scler.* (2012) 18:31–8. doi: 10.1177/1352458511416836
25. Schmierer K, Irlbacher K, Grosse P, Roricht S, Meyer BU. Correlates of disability in multiple sclerosis detected by transcranial magnetic stimulation. *Neurology.* (2002) 59:1218–24. doi: 10.1212/WNL.59.8.1218
26. Fuhr P, Kappos L. Evoked potentials for evaluation of multiple sclerosis. *Clin Neurophysiol.* (2001) 112:2185–9. doi: 10.1016/S1388-2457(01)00687-3
27. Fuhr P, Borggrefe-Chappuis A, Schindler C, Kappos L. Visual and motor evoked potentials in the course of multiple sclerosis. *Brain.* (2001) 124(Pt 11):2162–8. doi: 10.1093/brain/124.11.2162
28. Neva JL, Lakhani B, Brown KE, Wadden KP, Mang CS, Ledwell NH, et al. Multiple measures of corticospinal excitability are associated with clinical features of multiple sclerosis. *Behav Brain Res.* (2016) 297:187–95. doi: 10.1016/j.bbr.2015.10.015
29. Chaves AR, Kelly LP, Moore CS, Stefanelli M, Ploughman M. Prolonged cortical silent period is related to poor fitness and fatigue, but not tumor necrosis factor, in multiple sclerosis. *Clin Neurophysiol.* (2019) 130:474–83. doi: 10.1016/j.clinph.2018.12.015
30. Mori F, Kusayanagi H, Monteleone F, Moscatelli A, Nicoletti CG, Bernardi G, et al. Short interval intracortical facilitation correlates with the degree of disability in multiple sclerosis. *Brain Stimul.* (2013) 6:67–71. doi: 10.1016/j.brs.2012.02.001
31. Fuhr P, Agostino R, Hallett M. Spinal motor neuron excitability during the silent period after cortical stimulation. *Electroencephalogr Clin Neurophysiol.* (1991) 81:257–62. doi: 10.1016/0168-5597(91)90011-L
32. Ziemann U, Netz J, Szelenyi A, Homberg V. Spinal and supraspinal mechanisms contribute to the silent period in the contracting soleus muscle after transcranial magnetic stimulation of human motor cortex. *Neurosci Lett.* (1993) 156:167–71. doi: 10.1016/0304-3940(93)90464-V
33. Chen R, Lozano AM, Ashby P. Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. *Exp Brain Res.* (1999) 128:539–42. doi: 10.1007/s002210050878
34. Bertasi V, Bertolasi L, Frasson E, Priori A. The excitability of human cortical inhibitory circuits responsible for the muscle silent period after transcranial brain stimulation. *Exp Brain Res.* (2000) 132:384–9. doi: 10.1007/s002210000352
35. Shimizu T, Hino T, Komori T, Hirai S. Loss of the muscle silent period evoked by transcranial magnetic stimulation of the motor cortex in patients with cervical cord lesions. *Neurosci Lett.* (2000) 286:199–202. doi: 10.1016/S0304-3940(00)01125-3
36. Wu T, Sommer M, Tergau F, Paulus W. Modification of the silent period by double transcranial magnetic stimulation. *Clin Neurophysiol.* (2000) 111:1868–72. doi: 10.1016/S1388-2457(00)00426-0
37. Schnitzler A, Benecke R. The silent period after transcranial magnetic stimulation is of exclusive cortical origin: evidence from isolated cortical ischemic lesions in man. *Neurosci Lett.* (1994) 180:41–5. doi: 10.1016/0304-3940(94)90909-1
38. Caramia MD, Palmieri MG, Desiato MT, Boffa L, Galizia P, Rossini PM, et al. Brain excitability changes in the relapsing and remitting phases of multiple sclerosis: a study with transcranial magnetic stimulation. *Clin Neurophysiol.* (2004) 115:956–65. doi: 10.1016/j.clinph.2003.11.024
39. Nantes JC, Zhong J, Holmes SA, Narayanan S, Lapierre Y, Koski L. Cortical damage and disability in multiple sclerosis: relation to intracortical inhibition and facilitation. *Brain Stimul.* (2016) 9:566–73. doi: 10.1016/j.brs.2016.01.003
40. Mori F, Codeca C, Kusayanagi H, Monteleone F, Boffa L, Rimano A, et al. Effects of intermittent theta burst stimulation on spasticity in patients with multiple sclerosis. *Eur J Neurol.* (2010) 17:295–300. doi: 10.1111/j.1468-1331.2009.02806.x
41. Korzhova J, Bakulin I, Sinitsyn D, Poydasheva A, Suponeva N, Zakharova M, et al. High-frequency repetitive transcranial magnetic stimulation and intermittent theta-burst stimulation for spasticity management in secondary progressive multiple sclerosis. *Eur J Neurol.* (2019) 26:680–e44. doi: 10.1111/ene.13877
42. Weiss S, Mori F, Rossi S, Centonze D. Disability in multiple sclerosis: when synaptic long-term potentiation fails. *Neurosci Biobehav Rev.* (2014) 43:88–99. doi: 10.1016/j.neubiorev.2014.03.023
43. Zeller D, aufm Kampe K, Biller A, Stefan K, Gentner R, Schutz A, et al. Rapid-onset central motor plasticity in multiple sclerosis. *Neurology.* (2010) 74:728–35. doi: 10.1212/WNL.0b013e3181d31dcf
44. Wirsching I, Buttmann M, Odorfer T, Volkmann J, Classen J, Zeller D. Altered motor plasticity in an acute relapse of multiple sclerosis. *Eur J Neurosci.* (2018) 47:251–7. doi: 10.1111/ejn.13818
45. Stein RB, Everaert DG, Roy FD, Chong S, Soleimani M. Facilitation of corticospinal connections in able-bodied people and people with central nervous system disorders using eight interventions. *J Clin Neurophysiol.* (2013) 30:66–78. doi: 10.1097/WNP.0b013e31827ed6bd
46. Tomassini V, Johansen-Berg H, Jbabdi S, Wise RG, Pozzilli C, Palace J, et al. Relating brain damage to brain plasticity in patients with multiple sclerosis. *Neurorehabil Neural Repair.* (2012) 26:581–93. doi: 10.1177/1545968311433208
47. Tomassini V, Matthews PM, Thompson AJ, Fuglo D, Geurts JJ, Johansen-Berg H, et al. Neuroplasticity and functional recovery in multiple sclerosis. *Nat Rev Neurol.* (2012) 8:635–46. doi: 10.1038/nrneurol.2012.179
48. Schubert M, Wohlfarth K, Rollnik JD, Dengler R. Walking and fatigue in multiple sclerosis: the role of the

corticospinal system. *Muscle Nerve.* (1998) 21:1068–70. doi: 10.1002/(sici)1097-4598(199808)21:8<1068::aid-mus12>3.0.co;2-q
49. Everaert DG, Thompson AK, Chong SL, Stein RB. Does functional electrical stimulation for foot drop strengthen corticospinal connections? *Neurorehabil Neural Repair.* (2010) 24:168–77. doi: 10.1177/1545968309349939
50. Stein RB, Everaert D, Chong SL, Thompson AK. Using FES for foot drop strengthens cortico-spinal connections. In: *12th Annual Conference of teh International FES Society*. Philadelphia, PA (2007).
51. Lo AC, Triche EW. Improving gait in multiple sclerosis using robot-assisted, body weight supported treadmill training. *Neurorehabil Neural Repair.* (2008) 22:661–71. doi: 10.1177/1545968308318473
52. Giesser B, Beres-Jones J, Budovitch A, Herlihy E, Harkema S. Locomotor training using body weight support on a treadmill improves mobility in persons with multiple sclerosis: a pilot study. *Mult Scler.* (2007) 13:224–31. doi: 10.1177/1352458506070663
53. Charvet LE, Dobbs B, Shaw MT, Bikson M, Datta A, Krupp LB. Remotely supervised transcranial direct current stimulation for the treatment of fatigue in multiple sclerosis: Results from a randomized, sham-controlled trial. *Mult Scler.* (2018) 24:1760–69. doi: 10.1177/1352458517732842
54. Leocani L, Chieffo R, Gentile A, Centonze D. Beyond rehabilitation in MS: insights from non-invasive brain stimulation. *Mult Scler.* (2019) 25:1363–71. doi: 10.1177/1352458519865734
55. Ayache SS, Chalah MA. Transcranial direct current stimulation: a glimmer of hope for multiple sclerosis fatigue? *J Clin Neurosci.* (2018) 55:10–12. doi: 10.1016/j.jocn.2018.06.002
56. Ayache SS, Chalah MA. The place of transcranial direct current stimulation in the management of multiple sclerosis-related symptoms. *Neurodegener Dis Manag.* (2018) 8:411–22. doi: 10.2217/nmt-2018-0028
57. Palm U, Ayache SS, Padberg F, Lefaucheur JP. Non-invasive brain stimulation therapy in multiple sclerosis: a review of tDCS, rTMS and ECT results. *Brain Stimul.* (2014) 7:849–54. doi: 10.1016/j.brs.2014.09.014
58. Iodice R, Manganelli F, Dubbioso R. The therapeutic use of non-invasive brain stimulation in multiple sclerosis - a review. *Restor Neurol Neurosci.* (2017) 35:497–509. doi: 10.3233/RNN-170735
59. Nasios G, Bakirtzis C, Messinis L. Cognitive impairment and brain reorganization in MS: underlying mechanisms and the role of neurorehabilitation. *Front Neurol.* (2020) 11:147. doi: 10.3389/fneur.2020.00147
60. Abboud H, Hill E, Siddiqui J, Serra A, Walter B. Neuromodulation in multiple sclerosis. *Mult Scler.* (2017) 23:1663–76. doi: 10.1177/1352458517736150
61. Mori F, Codeca C, Kusayanagi H, Monteleone F, Buttari F, Fiore S, et al. Effects of anodal transcranial direct current stimulation on chronic neuropathic pain in patients with multiple sclerosis. *J Pain.* (2010) 11:436–42. doi: 10.1016/j.jpain.2009.08.011
62. Mori F, Nicoletti CG, Kusayanagi H, Foti C, Restivo DA, Marciani MG, et al. Transcranial direct current stimulation ameliorates tactile sensory deficit in multiple sclerosis. *Brain Stimul.* (2013) 6:654–9. doi: 10.1016/j.brs.2012.10.003
63. Turrigiano GG. Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci.* (1999) 22:221–7. doi: 10.1016/S0166-2236(98)01341-1
64. Ziemann U, Ilic TV, Pauli C, Meintzschel F, Ruge D. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. *J Neurosci.* (2004) 24:1666–72. doi: 10.1523/JNEUROSCI.5016-03.2004
65. Jung P, Ziemann U. Homeostatic and nonhomeostatic modulation of learning in human motor cortex. *J Neurosci.* (2009) 29:5597–604. doi: 10.1523/JNEUROSCI.0222-09.2009
66. Potter-Nerger M, Fischer S, Mastroeni C, Groppa S, Deuschl G, Volkmann J, et al. Inducing homeostatic-like plasticity in human motor cortex through converging corticocortical inputs. *J Neurophysiol.* (2009) 102:3180–90. doi: 10.1152/jn.91046.2008
67. Muller JF, Orekhov Y, Liu Y, Ziemann U. Homeostatic plasticity in human motor cortex demonstrated by two consecutive sessions of paired associative stimulation. *Eur J Neurosci.* (2007) 25:3461–8. doi: 10.1111/j.1460-9568.2007.05603.x
68. Wolpaw JR. What can the spinal cord teach us about learning and memory? *Neuroscientist.* (2010) 16:532–49. doi: 10.1177/1073858410368314
69. Karabanov A, Ziemann U, Hamada M, George MS, Quartarone A, Classen J, et al. Consensus paper: probing homeostatic plasticity of human cortex with non-invasive transcranial brain stimulation. *Brain Stimul.* (2015) 8:993–1006. doi: 10.1016/j.brs.2015.06.017
70. Mori F, Ljoka C, Magni E, Codeca C, Kusayanagi H, Monteleone F, et al. Transcranial magnetic stimulation primes the effects of exercise therapy in multiple sclerosis. *J Neurol.* (2011) 258:1281–7. doi: 10.1007/s00415-011-5924-1
71. Khaslavskaia S, Sinkjaer T. Motor cortex excitability following repetitive electrical stimulation of the common peroneal nerve depends on the voluntary drive. *Exp Brain Res.* (2005) 162:497–502. doi: 10.1007/s00221-004-2153-1
72. Khaslavskaia S, Ladouceur M, Sinkjaer T. Increase in tibialis anterior motor cortex excitability following repetitive electrical stimulation of the common peroneal nerve. *Exp Brain Res.* (2002) 145:309–15. doi: 10.1007/s00221-002-1094-9
73. Kido Thompson A, Stein RB. Short-term effects of functional electrical stimulation on motor-evoked potentials in ankle flexor and extensor muscles. *Exp Brain Res.* (2004) 159:491–500. doi: 10.1007/s00221-004-1972-4
74. Stein RB, Everaert DG, Thompson AK, Chong SL, Whittaker M, Robertson J, et al. Long-term therapeutic and orthotic effects of a foot drop stimulator on walking performance in progressive and nonprogressive neurological disorders. *Neurorehabil Neural Repair.* (2010) 24:152–67. doi: 10.1177/1545968309347681
75. Christiansen L, Perez MA. Targeted-Plasticity in the corticospinal tract after human spinal cord injury. *Neurotherapeutics.* (2018) 15:618–27. doi: 10.1007/s13311-018-0639-y
76. Bunday KL, Perez MA. Motor recovery after spinal cord injury enhanced by strengthening corticospinal synaptic transmission. *Curr Biol.* (2012) 22:2355–61. doi: 10.1016/j.cub.2012.10.046
77. Taylor JL, Martin PG. Voluntary motor output is altered by spike-timing-dependent changes in the human corticospinal pathway. *J Neurosci.* (2009) 29:11708–16. doi: 10.1523/JNEUROSCI.2217-09.2009
78. Suppa A, Quartarone A, Siebner H, Chen R, Di Lazzaro V, Del Giudice P, et al. The associative brain at work: evidence from paired associative stimulation studies in humans. *Clin Neurophysiol.* (2017) 128:2140–64. doi: 10.1016/j.clinph.2017.08.003
79. Mrachacz-Kersting N, Voigt M, Stevenson AJT, Aliakbaryhosseinabadi S, Jiang N, Dremstrup K, et al. The effect of type of afferent feedback timed with motor imagery on the induction of cortical plasticity. *Brain Res.* (2017) 1674:91–100. doi: 10.1016/j.brainres.2017.08.025
80. Thompson AK, Wolpaw JR. Restoring walking after spinal cord injury: operant conditioning of spinal reflexes can help. *Neuroscientist.* (2015) 21:203–15. doi: 10.1177/1073858414527541
81. Thompson AK, Wolpaw JR. Targeted neuroplasticity for rehabilitation. *Prog Brain Res.* (2015) 218:157–72. doi: 10.1016/bs.pbr.2015.02.002
82. Thompson AK, Wolpaw JR. Operant conditioning of spinal reflexes: from basic science to clinical therapy. *Front Integr Neurosci.* (2014) 8:25. doi: 10.3389/fnint.2014.00025
83. Carson RG, Kennedy NC. Modulation of human corticospinal excitability by paired associative stimulation. *Front Hum Neurosci.* (2013) 7:823. doi: 10.3389/fnhum.2013.00823
84. Palmer JA, Halter A, Gray W, Wolf SL, Borich MR. Modulatory effects of motor state during paired associative stimulation on motor cortex excitability and motor skill learning. *Front Hum Neurosci.* (2019) 13:8. doi: 10.3389/fnhum.2019.00008
85. Usrey WM, Reppas JB, Reid RC. Paired-spike interactions and synaptic efficacy of retinal inputs to the thalamus. *Nature.* (1998) 395:384–7. doi: 10.1038/26487
86. Bi GQ, Poo MM. Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *J Neurosci.* (1998) 18:10464–72. doi: 10.1523/JNEUROSCI.18-24-10464.1998
87. Mrachacz-Kersting N, Kristensen SR, Niazi IK, Farina D. Precise temporal association between cortical potentials evoked by motor imagination and afference induces cortical plasticity. *J Physiol.* (2012) 590:1669–82. doi: 10.1113/jphysiol.2011.222851

88. Mrachacz-Kersting N, Stevenson AJT, Jorgensen HRM, Severinsen KE, Aliakbaryhosseinabadi S, Jiang N, et al. Brain state-dependent stimulation boosts functional recovery following stroke. *Ann Neurol.* (2019) 85:84–95. doi: 10.1002/ana.25375
89. McSweeney FK, Murphy ES. *The Wiley Blackwell Handbook of Operant and Classical Conditioning.* Chichester, UK: John Wiley and Sons, Incorporated. (2014). doi: 10.1002/9781118468135
90. Schultz W. Neuronal reward and decision signals: from theories to data. *Physiol Rev*. (2015) 95:853–951. doi: 10.1152/physrev.00023.2014
91. Thompson AK, Fiorenza G, Smyth L, Favale B, Brangaccio J, Sniffen J. Operant conditioning of the motor-evoked potential and locomotion in people with and without chronic incomplete spinal cord injury. *J Neurophysiol.* (2019) 121:853–66. doi: 10.1152/jn.00557.2018
92. Thompson AK, Pomerantz FR, Wolpaw JR. Operant conditioning of a spinal reflex can improve locomotion after spinal cord injury in humans. *J Neurosci.* (2013) 33:2365–75. doi: 10.1523/JNEUROSCI.3968-12.2013
93. Wolpaw JR. The negotiated equilibrium model of spinal cord function. *J Physiol*. (2018) 596:3469–91. doi: 10.1113/JP275532
94. Thompson AK, Wolpaw JR. The simplest motor skill: mechanisms and applications of reflex operant conditioning. *Exerc Sport Sci Rev*. (2014) 42:82–90. doi: 10.1249/JES.0000000000000010
95. Chen XY, Wang Y, Chen Y, Chen L, Wolpaw JR. The inferior olive is essential for long-term maintenance of a simple motor skill. *J Neurophysiol.* (2016) 116:1946–55. doi: 10.1152/jn.00085.2016
96. Wolpaw JR, Chen XY. Operant conditioning of reflexes. In: Squire LR, editor. *Encyclopedia of Neuroscience*. Oxford: Academic Press (2009). p. 225–233. doi: 10.1016/B978-008045046-9.01347-4
97. Chen XY, Wolpaw JR. Probable corticospinal tract control of spinal cord plasticity in the rat. *J Neurophysiol.* (2002) 87:645–52. doi: 10.1152/jn.00391.2001
98. Chen XY, Wolpaw JR, Jakeman LB, Stokes BT. Operant conditioning of H-reflex in spinal cord-injured rats. *J Neurotrauma.* (1996) 13:755–66. doi: 10.1089/neu.1996.13.755
99. Thompson AK, Wolpaw JR. H-reflex conditioning during locomotion in people with spinal cord injury. *J Physiol*. (2019) doi: 10.1113/JP278173. [Epub ahead of print].
100. Wolpaw JR. Spinal cord plasticity in acquisition and maintenance of motor skills. *Acta Physiol.* (2007) 189:155–69. doi: 10.1111/j.1748-1716.2006.01656.x
101. Wolpaw JR. The complex structure of a simple memory. *Trends Neurosci.* (1997) 20:588–94. doi: 10.1016/S0166-2236(97)01133-8
102. Thompson AK, Chen XY, Wolpaw JR. Acquisition of a simple motor skill: task-dependent adaptation plus long-term change in the human soleus H-reflex. *J Neurosci.* (2009) 29:5784–92. doi: 10.1523/JNEUROSCI.4326-08.2009
103. Thompson AK, Cote RH, Sniffen JM, Brangaccio JA. Operant conditioning of the tibialis anterior motor evoked potential in people with and without chronic incomplete spinal cord injury. *J Neurophysiol.* (2018) 120:2745–60. doi: 10.1152/jn.00362.2018
104. Thompson AK, Favale BM, Velez J, Falivena P. Operant up-conditioning of the tibialis anterior motor-evoked potential in multiple sclerosis: feasibility case studies. *Neural Plast.* (2018) 2018:4725393. doi: 10.1155/2018/4725393
105. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. (1983) 33:1444–52. doi: 10.1212/WNL.33.11.1444
106. Vicars SM, Miguel CF, Sobie JL. Assessing preference and reinforcer effectiveness in dogs. *Behav Processes.* (2014) 103:75–83. doi: 10.1016/j.beproc.2013.11.006
107. Lee MS, Yu CT, Martin TL, Martin GL. On the relation between reinforcer efficacy and preference. *J Appl Behav Anal.* (2010) 43:95–100. doi: 10.1901/jaba.2010.43-95
108. Klose KJ, Needham BM, Schmidt D, Broton JG, Green BA. An assessment of the contribution of electromyographic biofeedback as an adjunct therapy in the physical training of spinal cord injured persons. *Arch Phys Med Rehabil.* (1993) 74:453–6. doi: 10.1016/0003-9993(93)90103-H
109. Brucker BS, Bulaeva NV. Biofeedback effect on electromyography responses in patients with spinal cord injury. *Arch Phys Med Rehabil.* (1996) 77:133–7. doi: 10.1016/S0003-9993(96)90157-4
110. Kohlmeyer KM, Hill JP, Yarkony GM, Jaeger RJ. Electrical stimulation and biofeedback effect on recovery of tenodesis grasp: a controlled study. *Arch Phys Med Rehabil.* (1996) 77:702–6. doi: 10.1016/S0003-9993(96)90011-8
111. Petrofsky JS. The use of electromyogram biofeedback to reduce Trendelenburg gait. *Eur J Appl Physiol.* (2001) 85:491–5. doi: 10.1007/s004210100466
112. van Dijk H, Jannink MJ, Hermens HJ. Effect of augmented feedback on motor function of the affected upper extremity in rehabilitation patients: a systematic review of randomized controlled trials. *J Rehabil Med.* (2005) 37:202–11. doi: 10.1080/16501970510030165
113. Devillard X, Rimaud D, Roche F, Calmels P. Effects of training programs for spinal cord injury. *Ann Readapt Med Phys.* (2007) 50:490–8:480–9. doi: 10.1016/j.annrmp.2007.04.013
114. Sapienza CM, Wheeler K. Respiratory muscle strength training: functional outcomes versus plasticity. *Semin Speech Lang.* (2006) 27:236–44. doi: 10.1055/s-2006-955114
115. S. Van Houtte Vanlandewijck Y, Gosselink R. Respiratory muscle training in persons with spinal cord injury: a systematic review. *Respir Med.* (2006) 100:1886–95. doi: 10.1016/j.rmed.2006.02.029
116. Gregory CM, Bowden MG, Jayaraman A, Shah P, Behrman A, Kautz SA, et al. Resistance training and locomotor recovery after incomplete spinal cord injury: a case series. *Spinal Cord.* (2007) 45:522–30. doi: 10.1038/sj.sc.3102002
117. Hicks AL, Martin KA, Ditor DS, Latimer AE, Craven C, Bugaresti J, et al. Long-term exercise training in persons with spinal cord injury: effects on strength, arm ergometry performance and psychological well-being. *Spinal Cord.* (2003) 41:34–43. doi: 10.1038/sj.sc.3101389
118. Hartkopp A, Harridge SD, Mizuno M, Ratkevicius A, Quistorff B, Kjaer M, et al. Effect of training on contractile and metabolic properties of wrist extensors in spinal cord-injured individuals. *Muscle Nerve.* (2003) 27:72–80. doi: 10.1002/mus.10290
119. Dragert K, Zehr EP. Bilateral neuromuscular plasticity from unilateral training of the ankle dorsiflexors. *Exp Brain Res.* (2011) 208:217–27. doi: 10.1007/s00221-010-2472-3
120. Chen XY, Chen Y, Wang Y, Thompson A, Carp JS, Segal RL, et al. Reflex conditioning: a new strategy for improving motor function after spinal cord injury. *Ann N Y Acad Sci.* (1198) (2010) 1198:E12–21. doi: 10.1111/j.1749-6632.2010.05565.x
121. Trompetto C, Buccolieri A, Marinelli L, Abbruzzese G. Differential modulation of motor evoked potential and silent period by activation of intracortical inhibitory circuits. *Clin Neurophysiol.* (2001) 112:1822–7. doi: 10.1016/S1388-2457(01)00644-7
122. Ashby P, Reynolds C, Wennberg R, Lozano AM, Rothwell J. On the focal nature of inhibition and facilitation in the human motor cortex. *Clin Neurophysiol.* (1999) 110:550–5. doi: 10.1016/S1388-2457(98)00082-0
123. Inghilleri M, Berardelli A, Cruccu G, Manfredi M. Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. *J Physiol.* (1993) 466:521–34.
124. Knash ME, Kido A, Gorassini M, Chan KM, Stein RB. Electrical stimulation of the human common peroneal nerve elicits lasting facilitation of cortical motor-evoked potentials. *Exp Brain Res.* (2003) 153:366–77. doi: 10.1007/s00221-003-1628-9
125. Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. *J Physiol.* (1996) 496 (Pt 3):873–81. doi: 10.1113/jphysiol.1996.sp021734
126. Di Lazzaro V, Ziemann U. The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. *Front Neural Circuits.* (2013) 7:18. doi: 10.3389/fncir.2013.00018
127. Nielsen JB, Tijssen MA, Hansen NL, Crone C, Petersen NT, Brown P, et al. Corticospinal transmission to leg motoneurones in human subjects with deficient glycinergic inhibition. *J Physiol.* (2002) 544(Pt 2):631–40. doi: 10.1113/jphysiol.22.024091
128. Boroojerdi B, Battaglia F, Muellbacher W, Cohen LG. Mechanisms influencing stimulus-response properties of the human corticospinal system. *Clin Neurophysiol.* (2001) 112:931–7. doi: 10.1016/S1388-2457(01)00523-5
129. Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W. The effect of lorazepam on the motor cortical excitability in man. *Exp Brain Res.* (1996) 109:127–35. doi: 10.1007/BF00228633

130. V. Di Lazzaro Oliviero A, Meglio M, Cioni B, Tamburrini G, Tonali P, Rothwell JC. Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. *Clin Neurophysiol.* (2000) 111:794–9. doi: 10.1016/S1388-2457(99)00314-4
131. Siebner HR, Dressnandt J, Auer C, Conrad B. Continuous intrathecal baclofen infusions induced a marked increase of the transcranially evoked silent period in a patient with generalized dystonia. *Muscle Nerve.* (1998) 21:1209–12. doi: 10.1002/(sici)1097-4598(199809)21:9<1209::aid-mus15>3.0.co;2-m
132. Tataroglu C, Genc A, Idiman E, Cakmur R, Idiman F. Cortical silent period and motor evoked potentials in patients with multiple sclerosis. *Clin Neurol Neurosurg.* (2003) 105:105–10. doi: 10.1016/S0303-8467(02)00127-0
133. McDonnell MN, Orekhov Y, Ziemann U. The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Exp Brain Res.* (2006) 173:86–93. doi: 10.1007/s00221-006-0365-2
134. Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *J Physiol.* (1999) 517:591–7. doi: 10.1111/j.1469-7793.1999.0591t.x
135. Mrachacz-Kersting N, Kersting UG, de Brito Silva P, Makihara Y, Arendt-Nielsen L, Sinkjaer T, et al. Acquisition of a simple motor skill: task-dependent adaptation and long-term changes in the human soleus stretch reflex. *J Neurophysiol.* (2019) 122:435–46. doi: 10.1152/jn.00211.2019
136. Makihara Y, Segal RL, Wolpaw JR, Thompson AK. Operant conditioning of the soleus H-reflex does not induce long-term changes in the gastrocnemius H-reflexes and does not disturb normal locomotion in humans. *J Neurophysiol.* (2014) 112:1439–46. doi: 10.1152/jn.00225.2014
137. Milinis K, Tennant A, Young CA, TONIC study group. Spasticity in multiple sclerosis: Associations with impairments and overall quality of life. *Mult Scler Relat Disord.* (2016) 5:34–9. doi: 10.1016/j.msard.2015.10.007
138. Nielsen J, Petersen N, Crone C. Changes in transmission across synapses of Ia afferents in spastic patients. *Brain.* (1995) 118:995–1004. doi: 10.1093/brain/118.4.995
139. Crone C, Nielsen J, Petersen N, Ballegaard M, Hultborn H. Disynaptic reciprocal inhibition of ankle extensors in spastic patients. *Brain.* (1994) 117:1161–8. doi: 10.1093/brain/117.5.1161
140. Morita H, Crone C, Christenhuis D, Petersen NT, Nielsen JB. Modulation of presynaptic inhibition and disynaptic reciprocal Ia inhibition during voluntary movement in spasticity. *Brain.* (2001) 124(Pt 4):826–37. doi: 10.1093/brain/124.4.826
141. Chen Y, Chen L, Wang Y, Wolpaw JR, Chen XY. Persistent beneficial impact of H-reflex conditioning in spinal cord-injured rats. *J Neurophysiol.* (2014) 112:2374–81. doi: 10.1152/jn.00422.2014
142. Chen Y, Chen L, Liu R, Wang Y, Chen XY, Wolpaw JR. Locomotor impact of beneficial or nonbeneficial H-reflex conditioning after spinal cord injury. *J Neurophysiol.* (2014) 111:1249–58. doi: 10.1152/jn.00756.2013
143. Thompson AK, Wolpaw JR. Operant conditioning of spinal reflexes to improve motor function after spinal cord injury. In: Fehlings MG, Vaccaro A, Boakye RS, Burns A, Di Tunno J, editors. *Essentials of Spinal Cord Injury*. New York, NY: Thieme Publishers. (2012). p. 545–557.
144. Chen Y, Chen XY, Jakeman LB, Chen L, Stokes BT, Wolpaw JR. Operant conditioning of H-reflex can correct a locomotor abnormality after spinal cord injury in rats. *J Neurosci.* (2006) 26:12537–43. doi: 10.1523/JNEUROSCI.2198-06.2006
145. Segal RL, Wolf SL. Operant conditioning of spinal stretch reflexes in patients with spinal cord injuries. *Exp Neurol.* (1994) 130:202–13. doi: 10.1006/exnr.1994.1199
146. Chalah MA, Kauv P, Creange A, Hodel J, Lefaucheur JP, Ayache SS. Neurophysiological, radiological and neuropsychological evaluation of fatigue in multiple sclerosis. *Mult Scler Relat Disord.* (2019) 28:145–52. doi: 10.1016/j.msard.2018.12.029
147. Dario A, Scamoni C, Picano M, Casagrande F, Tomei G. Pharmacological complications of the chronic baclofen infusion in the severe spinal spasticity. Personal experience and review of the literature. *J Neurosurg Sci.* (2004) 48:177–81.
148. Dario A, Tomei G. A benefit-risk assessment of baclofen in severe spinal spasticity. *Drug Saf.* (2004) 27:799–818. doi: 10.2165/00002018-200427110-00004
149. Shi J, Wu X, Chen Y. Study on Dalfampridine in the treatment of multiple sclerosis mobility disability: a meta-analysis. *PLoS ONE.* (2019) 14:e0222288. doi: 10.1371/journal.pone.0222288
150. Dunn J, Blight A. Dalfampridine: a brief review of its mechanism of action and efficacy as a treatment to improve walking in patients with multiple sclerosis. *Curr Med Res Opin.* (2011) 27:1415–23. doi: 10.1185/03007995.2011.583229
151. Applebee A, Goodman AD, Mayadev AS, Bethoux F, Goldman MD, Klingler M, et al. Effects of Dalfampridine extended-release tablets on 6-minute walk distance in patients with multiple sclerosis: a post hoc analysis of a double-blind, placebo-controlled trial. *Clin Ther.* (2015) 37:2780–7. doi: 10.1016/j.clinthera.2015.10.014
152. Goodman AD, Brown TR, Krupp LB, Schapiro RT, Schwid SR, Cohen R, et al. Sustained-release oral fampridine in multiple sclerosis: a randomised, double-blind, controlled trial. *Lancet.* (2009) 373:732–8. doi: 10.1016/S0140-6736(09)60442-6
153. Nicoletti CG, Monteleone F, Marfia GA, Usiello A, Buttari F, Centonze D, et al. Oral D-Aspartate enhances synaptic plasticity reserve in progressive multiple sclerosis. *Mult Scler.* (2020) 26:304–11. doi: 10.1177/1352458519828294
154. Ferrucci R, Vergari M, Cogiamanian F, Bocci T, Ciocca M, Tomasini E, et al. Transcranial direct current stimulation (tDCS) for fatigue in multiple sclerosis. *NeuroRehabilitation.* (2014) 34:121–7. doi: 10.3233/NRE-13 1019

Conflict of Interest: AT holds several patents related to operant conditioning of spinal reflexes (US patent number 8862236, 9138579, and 9545515). These patents are not providing income in any form.

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

frontiers in Neurology

ORIGINAL RESEARCH
published: 24 July 2020
doi: 10.3389/fneur.2020.00735

Validation of Quantitative Scores Derived From Motor Evoked Potentials in the Assessment of Primary Progressive Multiple Sclerosis: A Longitudinal Study

Martin Hardmeier[1]*, *Christian Schindler*[2], *Jens Kuhle*[1] and *Peter Fuhr*[1]

[1] Department of Neurology, University Hospital Basel, University of Basel, Basel, Switzerland, [2] Swiss Tropical and Public Health Institute (Swiss TPH), University of Basel, Basel, Switzerland

Objective: To evaluate the sensitivity to change of differently calculated quantitative scores from motor evoked potentials (MEP) in patients with primary progressive multiple sclerosis (PPMS).

Methods: Twenty patients with PPMS had MEP to upper and lower limbs at baseline, years 1 and 2 measured in addition to clinical assessment [Expanded Disability Status Scale (EDSS), ambulation score]; a subsample ($n = 9$) had a nine-hole peg test (NHPT) and a timed 25-foot walk (T25FW). Quantitative MEP scores for upper limbs (qMEP-UL), lower limbs (qMEP-LL), and all limbs (qMEP) were calculated in three different ways, based on *z*-transformed central motor conduction time (CMCT), shortest corticomuscular latency (CxM-sh), and mean CxM (CxM-mn). Changes in clinical measures and qMEP metrics were analyzed by repeated-measures analysis of variance (rANOVA), and a factor analysis was performed on change in qMEP metrics.

Results: Expanded Disability Status Scale and ambulation score progressed in the rANOVA model ($p < 0.05$; *post-hoc* comparison baseline–year 2, $p < 0.1$). Lower limb and combined qMEP scores showed significant deterioration of latency ($p < 0.01$, MEP-LL_CxM-sh: $p < 0.05$) and in *post-hoc* comparisons (baseline–year 2, $p < 0.05$), qMEP_CxM-mn even over 1 year ($p < 0.05$). Effect sizes were higher for qMEP scores than for clinical measures, and slightly but consistently higher when based on CxM-mn compared to CxM-sh or CMCT. Subgroup analysis yielded no indication of higher sensitivity of timed clinical measures over qMEP scores. Two independent factors were detected, the first mainly associated with qMEP-LL, the second with qMEP-UL, explaining 65 and 29% of total variability, respectively.

Conclusions: Deterioration in qMEP scores occurs earlier than EDSS progression in patients with PPMS. Upper and lower limb qMEP scores contribute independently to measuring change, and qMEP scores based on mean CxM are advantageous. The capability to detect subclinical changes longitudinally is a unique property of EP and complementary to clinical assessment. These features underline the role of EP as candidate biomarkers to measure effects of therapeutic interventions in PPMS.

Keywords: motor evoked potentials (MEP), primary progressive multiple sclerosis (PPMS), quantitative EP score, biomarker, longitudinal study

OPEN ACCESS

Edited by:
Ulrich Palm,
Medical Park Chiemseeblick, Germany

Reviewed by:
Xavier Moisset,
INSERM U1107 Douleur et Biophysique Neurosensorielle (Neuro-Dol), France
Grigorios Nasios,
University of Ioannina, Greece
Rosa Iodice,
University of Naples Federico II, Italy

***Correspondence:**
Martin Hardmeier
martin.hardmeier@usb.ch

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *03 February 2020*
Accepted: *15 June 2020*
Published: *24 July 2020*

Citation:
Hardmeier M, Schindler C, Kuhle J and Fuhr P (2020) Validation of Quantitative Scores Derived From Motor Evoked Potentials in the Assessment of Primary Progressive Multiple Sclerosis: A Longitudinal Study. Front. Neurol. 11:735. doi: 10.3389/fneur.2020.00735

INTRODUCTION

Development of therapies in primary progressive multiple sclerosis (PPMS) is hampered by the fact that detecting disease progression by clinical assessment needs considerable sample sizes and follow-up time to be meaningful (1, 2). Biomarkers allowing shorter multicenter clinical trials in small patient groups are not well-established (3, 4), and several candidate biomarkers have been proposed, including evoked potentials (EPs) (5).

Evoked potentials yield complementary information to clinical assessment as they are closely related to demyelination and measure subclinical changes, which may transform only later into clinical disability. Animal models have not only shown close correlations between demyelination and latency delay (6), but also between the recovery of delayed latencies with remyelination, bidirectionally paralleled by clinical function (7, 8). Several clinical studies have reported that scores from multimodal EP are predictive of disease course in relapsing and progressive multiple sclerosis (MS) [review in (9)], and short-term test–retest variability is reasonably low for quantitative EP scores (qEPS) (10). Longitudinal EP studies, which evaluate sensitivity to change of EP scores, are scarce in PPMS. In one small study, a multimodal qEPS deteriorated after 6 months, whereas the Expanded Disability Status Scale (EDSS) became significantly worse only after 12 months (11).

Motor evoked potentials (MEPs) to upper and lower limbs are an essential part of a multimodal EP assessment. Out of several measures derived from MEPs, latency is most closely linked to abnormal signal conduction in the corticospinal tract and a robust and easily registered MEP component (12). For diagnostic purposes, it is recommended to use the central motor conduction time (CMCT), which is specific for abnormalities in central signal conduction (13). However, test–retest reliability of CMCT is lower as compared to corticomuscular latency (CxM) (10), making CxM probably better suited to monitor disease course, provided that peripheral nerve disease has been excluded beforehand.

From a pathophysiological point of view, both latency delay and variability of MEP onset are features of disturbed signal propagation (14). In MS, onset latencies have been shown to be significantly more variable than in healthy controls and independent of latency delay (15). Moreover, the dispersion of MEP responses has been included in a semiquantitative EP scoring system (16). To account for onset variability and latency in one number, we currently calculated the mean CxM (CxM-mn), which is close to the shortest CxM (CxM-sh) in case of low variability and markedly longer in the case of high variability.

In the current study, we aim to scrutinize the MEP component of the multimodal qEPS regarding sensitivity to change in an independent sample of patients with PPMS and to determine the optimal way of its calculation.

For this purpose, we calculated qMEP scores based on CMCT, CxM-sh, and CxM-mn for upper limbs (qMEP-UL), lower limbs (qMEP-LL), and the combination of both (qMEP); evaluated longitudinal change of these nine qMEP metrics, as well as of clinical measures; and performed a factor analysis to determine the contribution of the different qMEP metrics to measuring change in latencies.

METHODS

Subjects

Twenty subjects with PPMS had MEP and clinical assessment at baseline years 1 and 2. Inclusion criteria were aged between 18 and 65 years and a primary progressive disease course as defined in the 2017 revisions of the McDonald criteria (17). Exclusion criteria comprised contraindications to MEP recording (epilepsy, moveable metal implants, pacemaker, pregnancy), inability to provide informed consent, and the presence of other diseases than MS interfering with MEP recording. All patients gave written informed consent in accordance with the Declaration of Helsinki.

Clinical Assessment

Patients were examined at least annually at our MS center by certified physicians using the EDSS (18) as defined in Neurostatus (19). Neurostatus includes an ambulation score ranging from 0 (unrestricted) to 12 (restricted to bed or chair, EDSS 8.0), which differs from the EDSS in a more granular representation of EDSS steps 6.0 and 6.5, where the ambulation scores are 5 to 7 and 8 to 9, respectively, taking walking distance and kind of walking aid used into account (see **Supplemental Material**). However, EDSS steps 0 to 4.0 are only represented as ambulation scores 0 to 1. All EDSS scores were checked for congruency with rating of functional systems and ambulation.

In a subsample, a nine-hole peg test (NHPT) as a timed measure of dexterity and a timed 25-foot walk (T25FW) as a timed measure of ambulation were available. They were performed according to the standards described in the Multiple Sclerosis Functional Composite [*z*-transformed relative to the NMSS sample (20)].

MEP Assessment

All MEPs to upper and lower limbs were recorded in our laboratory (Department of Neurology Hospital of the University of Basel) according to internal standards closely following the recommendations of the International Federation of Clinical Neurophysiology (IFCN) (13). Our clinical protocol is optimized for reproducibility and time efficiency using parasagittal stimulation with a round coil (MagProCompact, C-100, coil diameter 12.5 cm; Magventure Farum, Denmark; or Magstim 200, coil diameter 14 cm; The Magstim Company; Whitland, Wales, Great Britain) for upper and lower limbs at 80 to 100% stimulator output. Facilitation is achieved by slight contraction of the target muscles (m. abductor digiti minimi for upper limbs, m. tibialis anterior for lower limbs); for the spinomuscular latency, magnetic stimulation over the spine (cervical vertebra 7; lumbar vertebra 5) is applied. Cortical stimulation comprises eight stimuli (four coil side A, four coil side B), spinal stimulation four stimuli (two with coil side A, two with coil side B), recorded bilaterally resulting in eight cortical and four spinal responses per side.

All MEP curves were exported from the recording machine and uploaded to EPMark, a software tool for standardized EP reading. All curves were rated by a single rater (M.H.); follow-up curves were rated in comparison to baseline examinations to reduce inconsistencies due to curve rating.

Motor evoked potentials were analyzed for each side and limb and calculated in three ways based on the shortest CxM (CxM-sh), the mean CxM (CxM-mn), and the CMCT (difference between the CxM-sh and shortest spinomuscular latency). Mean CxM was calculated only if at least three of eight responses were available. In one patient, the unrecordable year 1 values of lower limb MEP were replaced by the baseline values. All CxM and CMCT values were z-transformed and corrected for height in lower limbs (see **Supplemental Material**), z-values from left and right sides were averaged to yield a one number score for upper limbs (qMEP-UL), lower limbs (qMEP-LL), and all limbs (qMEP) for each mode of calculation.

Statistical Analysis

Statistical analyses were conducted using SPSS Version 22 (IBM Corporation, Armonk, NY, USA). Repeated-measures analysis of variance (ANOVA) was used to compare means of qMEP metrics and clinical assessments longitudinally expressing the degree of temporal variation by partial η^2. *Post-hoc* comparisons between pairs of time points were conducted using Bonferroni correction. For each MEP index and each patient, the three consecutive measurements were summarized by an average linear slope [slope = (x(year 2) – x(year 0))/2] and a non-linear trend [trend = (x(year 2) – 2*x(year 1) + x(year 0))]. A factor analysis using principal component analysis followed by Varimax rotation was run on the two parameters across the nine qMEP metrics. For a sensitivity analysis of the NHPT, a paired t-test was run on all subjects with at least two assessments of the NHPT (see **Supplemental Material**).

RESULTS

Subjects had a mean age of 51.3 years (SD = 7.9) and a disease duration of 8.2 (SD = 6.7) years. The mean time between baseline and year 1 as well as year 2 assessments was 0.99 (SD = 0.12) and 2.1 (SD = 0.14) years, respectively. Median EDSS at baseline was 3.75 (range = 2.0–6.5), and median ambulation score 1 (0–9). Mean and standard deviation (SD) of qMEP metrics are given in the fifth column of **Table 1**.

At baseline, a subsample of patients had assessments of NHPT ($n = 13$) and T25FW ($n = 9$), of whom nine subjects had NHPT and T25FW at all three time points.

The results of the repeated-measures ANOVA are given in **Table 1**; p-values relate to the linear contrasts. EDSS and ambulation score progressed over time ($p < 0.05$), with a non-significant change over the 2 year period ($p < 0.1$ after Bonferroni correction). Latency increased significantly in qMEP-LL ($p < 0.01$ for CxM-mn and CMCT, $p < 0.05$ for CxM-sh) and combined qMEP scores (all $p < 0.01$), and the increase in the qMEP-CxM_mn score being statistically significant even in the first year ($p < 0.05$), as depicted in **Figure 1**. Effect sizes were higher in qMEP-LL and combined qMEP scores than in clinical assessments, and highest in scores based on CxM_mn. QMEP-UL did not significantly change over time.

Subgroup analysis (**Table 2**) in subjects ($n = 9$) with complete assessments of the NHPT and the T25FW showed a similar pattern. Whereas, changes in clinical measures were not significant (ambulation: $p < 0.1$; others $p > 0.1$), qMEP-LL_CxM-mn, qMEP_CxM-mn, and qMEP_CxM-sh showed statistically significant deterioration with highest effect sizes for measures calculated from CxM-mn. The sensitivity analysis of the NHPT (**Supplemental Material**) based on subjects with a baseline and a year 2 examination ($n = 12$) yielded a comparable non-significant change for the NHPT ($p = 0.06$) and the qMEP-UL_CxM-mn ($p = 0.1$).

TABLE 1 | Analysis of longitudinal change in EDSS, ambulation and qMEP scores.

		$F_{(2,38)}$	p-value	Effect size	Mean y0	Change y1-y0	Change y2-y1	Change y2-y0
Clinical	EDSS	3.278	**<0.05**	0.147	3.9 (1.2)	0.18 (−0.23 to 0.58)	0.33 (−0.25 to 0.90)	0.50 (−0.06 to 1.06) ^
	Ambulation	4.499	**<0.05**	0.191	1.9 (2.2)	0.70 (−0.11 to 1.51)	0.55 (−0.55 to 1.65)	1.25 (−0.07 to 2.57) ^
qMEPUL	CMCT	1.773	n.s.	0.085	3.62 (2.85)	0.05 (−1.04 to 1.13)	0.75 (−0.57 to 2.07)	0.80 (−0.53 to 2.13)
	CxM_sh	1.575	n.s.	0.077	5.13 (2.95)	0.04 (−071 to 0.79)	0.49 (−0.43 to 1.40)	0.52 (−0.39 to 1.43)
	CxM_mn	2.178	n.s.	0.103	4.33 (3.87)	0.15 (−0.57 to 0.86)	0.51 (−0.35 to 1.37)	0.66 (−0.35 to 1.67)
qMEPLL	CMCT	5.468	**<0.01**	0.223	5.24 (5.04)	0.98 (−0.42 to 2.38)	0.86 (−0.56 to 2.29)	**1.84 (0.29 to 3.40) ***
	CxM_sh	4.588	**<0.05**	0.195	7.04 (5.23)	0.74 (−0.25 to 1.74)	0.54 (−0.55 to 1.62)	**1.28 (0.03 to 2.54) ***
	CxM_mn	5.832	**<0.01**	0.235	7.67 (7.05)	0.75 (−0.13 to 1.62)	0.61 (−0.38 to 1.60)	**1.36 (0.12 to 2.60) ***
qMEP	CMCT	6.285	**<0.01**	0.249	4.43 (3.61)	0.51 (−0.37 to 1.40)	0.81 (−0.23 to 1.84)	**1.32 (0.29 to 2.36) ***
	CxM_sh	5.422	**<0.01**	0.222	5.92 (3.64)	0.39 (−0.22 to 1.00)	0.51 (−0.24 to 1.27)	**0.90 (0.12 to 1.69) ***
	CxM_mn	7.530	**<0.01**	0.284	6.00 (5.09)	**0.61 (0.07 to 1.15) ***	0.56 (−0.20 to 1.33)	**1.17 (0.17 to 2.17) ***

*Univariate repeated measures ANOVA (n = 20) and post-hoc paired comparisons for EDSS and ambulation score as well as qMEP scores calculated from upper limbs (qMEP-UL), lower limbs (qMEP-LL), and the combination of both (qMEP) based on central motor conduction time (CMCT), shortest cortico-muscular latency (CxM_sh), and mean CxM (CxM_mn). QMEP-scores are given as the sum of z-transformed latencies divided by the number of limbs examined. For all variables, F-values, p-values (linear contrast), and effect sizes are given along with their mean values and standard deviations (SD) at baseline (y0), and their mean changes between different years, with 95% confidence intervals (95%CI). ^p < 0.1; *p < 0.05, with Bonferroni correction for multiple comparisons. Significant values are given in bold.*

Factor analysis (**Table 3**) showed that qMEP-UL and qMEP-LL provide complementary information for the detection of longitudinal change in MEP onset latency, regardless whether the parameter of change was the linear slope or a non-linear trend (**Figure 2**). The first factor was mainly determined by qMEP-LL, and the second factor mainly by qMEP-UL explaining 65 and 29% of total variability. The combined qMEP scores load on both factors in a balanced way.

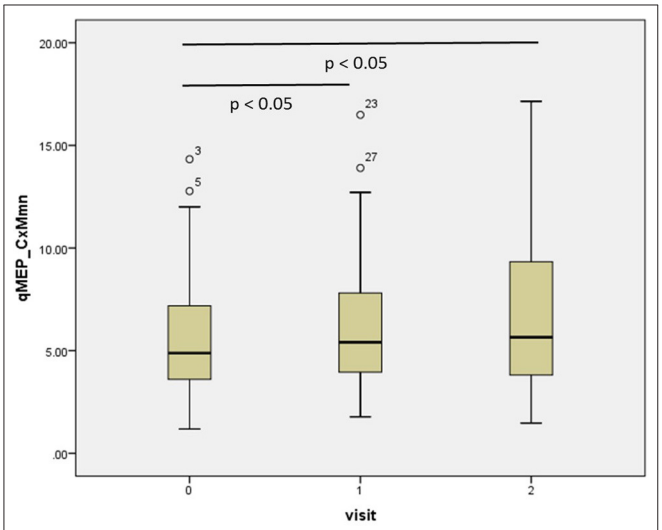


FIGURE 1 | Boxplot diagram showing the distribution of the combined quantitative MEP score based on the mean corticomuscular latency (qMEP_CxM-mn) at baseline, years 1 and 2. *P*-values are given for pairwise *post-hoc* comparison after Bonferroni correction.

DISCUSSION

In the current study, we examined 20 patients with primary progressive MS longitudinally over 2 years to scrutinize qMEP scores regarding sensitivity to change and to determine the optimal way of calculating the qMEP. In parallel to clinical progression as measured by EDSS and ambulation score over 2 years, lower limbs and combined qMEP scores indicated significant deterioration of latency delays with higher effect sizes than the EDSS and ambulation score. Differences between differently calculated qMEP scores were small, albeit scores based on mean CxM had highest effect sizes throughout, and only the combined qMEP score based on mean CxM showed a significant deterioration already in the first year. Moreover, in a subgroup analysis, timed clinical assessments did not show higher sensitivity than qMEP scores. Two independent factors were detected, the first mainly associated with qMEP-LL, the second one with qMEP-UL, explaining 65 and 29% of total variability, respectively. Upper and lower limb qMEPs contribute to the combined qMEP score in a balanced way.

Our main finding is that increases in latency delays over 2 years, as measured by lower limb and combined qMEP scores, were stronger in terms of effect size than increases in disability as measured by EDSS. Moreover, significant deterioration in the first year was observed in the combined qMEP based on mean CxM, but not in any of the clinical parameters. This result replicates the principal findings of a previous study in PPMS (11) in an independent sample of patients and is in line with several EP studies showing deterioration of EP scores over time in samples with relapsing remitting MS, as well as samples with relapsing and progressive MS [review in (9)]. In the former PPMS study, a multimodal qEPS changed already after 6 months, whereas the EDSS deteriorated only after 1 year (11). The higher temporal dynamics are most likely due to the faster

TABLE 2 | Subgroup analysis of longitudinal change in T25FW and NHPT.

		$F_{(2,16)}$	*p*-value	Effect size	Mean y0	Change y1-y0	Change y2-y1	Change y2-y0
Clinical	EDSS	2.266	n.s.	0.147	3.94 (1.13)	−0.28 (−0.85 to 0.29)	0.78 (−0.53 to 2.09)	0.50 (−0.81 to 1.81)
	Ambulation	3.653	<0.1	0.191	1.89 (1.83)	0.44 (−0.58 to 1.46)	1.44 (−0.87 to 3.75)	1.89 (−0.97 to 4.75)
	zT25FW	2.048	n.s.	0.204	9.18 (8.12)	−0.05 (−0.45 to 0.34)	−1.27 (−3.82 to 1.23)	−1.32 (−4.18 to 1.55)
	zNHPT	1.065	n.s.	0.118	−0.74 (1.05)	0.07 (−0.24 to 0.38)	−0.26 (−0.99 to 0.47)	−0.19 (−0.76 to 0.37)
qMEPUL	CMCT	1.727	n.s.	0.178	4.00 (5.21)	0.32 (−1.43 to 2.06)	1.11 (−1.44 to 3.66)	1.43 (−1.45 to 4.31)
	CxM_sh	1.726	n.s.	0.177	3.43 (3.52)	0.22 (−0.99 to 1.43)	0.77 (−0.99 to 2.53)	0.99 (−1.00 to 2.99)
	CxM_mn	2.056	n.s.	0.204	4.83 (3.49)	0.44 (−0.74 to 1.61)	0.66 (−0.91 to 2.22)	1.10 (−0.96 to 3.14)
qMEPLL	CMCT	2.474	n.s.	0.236	7.00 (6.5)	0.11 (−2.34 to 2.55)	1.52 (−1.15 to 4.19)	1.63 (−0.66 to 3.92)
	CxM_sh	2.668	n.s.	0.250	4.65 (4.71)	0.14 (−1.54 to 1.83)	1.11 (−0.84 to 3.06)	1.26 (−0.48 to 3.00)
	CxM_mn	4.123	**<0.05**	0.340	6.43 (5.04)	0.34 (−1.17 to 1.85)	1.27 (−0.46 to 2.99)	1.61 (−0.45 to 3.66)
qMEP	CMCT	3.558	<0.1	0.308	5.63 (5.46)	1.47 (−0.48 to 3.43)	−0.06 (−1.71 to 1.60)	1.42 (−0.62 to 3.45)
	CxM_sh	4.489	**<0.05**	0.359	4.07 (3.61)	−0.01 (−1.02 to 1.00)	1.06 (−0.12 to 2.23)	1.05 (−0.40 to 2.49)
	CxM_mn	5.629	**<0.05**	0.413	5.25 (3.49)	0.60 (−0.28 to 1.49)	1.03 (−0.25 to 2.31)	1.63 (−0.42 to 3.68)

Univariate repeated measures ANOVA and post-hoc paired comparisons on all patients with complete T25FW and NHPT (n = 9) for EDSS, ambulation score, z-transformed timed 25 foot walk (zT25FW) and z-transformed nine hole peg test (zNHPT) as well as qMEP scores calculated from upper limbs (qMEP-UL), lower limbs (qMEP-LL), and the combination of both (qMEP) based on central motor conduction time (CMCT), shortest cortico-muscular latency (CxM_sh), and mean CxM (CxM_mn). For all variables, F-values, p-values (linear contrast), and effect sizes are given along with their mean values and standard deviations (SD) at baseline (y0), and their mean changes between different years with 95% confidence intervals (95%CI). Significant values are given in bold. All post-hoc comparisons were non-significant.

TABLE 3 | Factor analysis of longitudinal change in qMEP metrics.

		Linear contrasts		Non-linear contrasts	
		Factor 1	**Factor 2**	**Factor 1**	**Factor 2**
Model	Eigenvalue	5.876	2.610	5.945	2.608
	Explained variance %	65.3	29.0	66.1	29.0
Factor loadings					
qMEPUL	CMCT	0.065	0.986	0.089	0.983
	CxM_sh	0.196	0.920	0.081	0.986
	CxM_mn	0.019	0.988	0.035	0.969
qMEPLL	CMCT	0.980	0.001	0.965	0.166
	CxM_sh	0.991	−0.031	0.994	−0.008
	CxM_mn	0.965	0.029	0.991	−0.010
qMEP	CMCT	0.797	0.433	0.777	0.605
	CxM_sh	0.829	0.545	0.782	0.607
	CxM_mn	0.749	0.634	0.529	0.709

Factor analysis of linear and quadratic contrasts of temporal change in qMEP metrics defined at the individual patient level using principal component and Varimax rotation. Eigenvalues, explained variance and factor loadings are given. Both individual contrasts revealed two independent dimensions (factors). The loading matrices show that the two factors of each contrast are largely determined by the lower and upper limb measurements, respectively.

clinical progression in the previous sample. Additionally, the applied multimodal qEPS includes motor, somatosensory, and visual EP, which probably increases the sensitivity to change. As individual patients are likely to deteriorate in different functional systems at different pace, a multimodal EP score is more likely to capture changes than a single modality. However, it remains to be determined whether the different EP modalities are equally sensitive to change.

In a recent cross-sectional study, MEPs from upper limbs only have been proposed as an outcome measure in clinical trials in patients with progressive forms of MS (21). The authors argue that lower limb MEPs are frequently absent and do not contribute to measuring deterioration. However, patients had considerable disability with a mean EDSS of 5.8, and the majority had a secondary progressive MS. In contrast, the current longitudinal analysis in less disabled patients with primary progressive MS clearly shows the high contribution of lower limb involvement to disease progression. Furthermore, upper and lower limb qMEP scores contribute independently to measuring disease progression. These results favor the use of a combined qMEP score, at least in patients with comparable disease characteristics and disability.

Variability of onset latencies is a physiological phenomenon and most likely due to short-term fluctuations in cortical and spinal excitability (13, 22). In MS, reliability of signal conduction is reduced in demyelinated tracts (14) due to less accurate temporal summation at the convergence of corticospinal axons in the spinal motoneuron. Significantly increased variability of MEP onset as quantified by the mean consecutive difference between several stimuli has been found in patients with MS independent of latency delay (15). However, in the current factor analysis, we could not detect an independent contribution of mean CxM, indicating that onset variability may not add to detection of change. Our approach may have been less sensitive than the mean consecutive difference, which, on the downside, poses other problems when used in a score, as it is an additional metric and a relative measure.

The slightly but consistently better performance of mean CxM over shortest CxM and CMCT may be related to its statistical properties with higher test–retest reliability (10) because an averaged response is a more robust estimate than a maximal response. However, the closer relationship to pathophysiology by inclusion of the variability of the onset latencies may also play a role.

To increase the sensitivity of clinical assessment for detecting progression, a combination of the EDSS with timed examinations as the NHPT and T25FW has been proposed in progressive MS (23, 24). There are only a few studies that compared EP with timed clinical assessment. Upper limb MEP correlated with the NHPT (21) and lower limb MEP with T25FW (25) cross-sectionally. Balance problems were more closely related to tibial somatosensory EP than to lower limb MEP (26). In the current study, we had only a small subsample to compare timed clinical measures to qMEP scores longitudinally. In these patients, we found no evidence indicating that NHPT or T25FW was superior to qMEP scores. However, the present sample size is too small to draw firm conclusions. Larger scaled studies are needed to better characterize the comparative sensitivity to change of timed clinical assessments and EP scores from different modalities.

Generally, clinical assessment and EP differ in their content validity. Expanded Disability Status Scale, NHPT, and T25FW measure global clinical function, dexterity, and walking capability, respectively (18, 27, 28). They are influenced by day-to-day fluctuations in performance, as well as imprecision of the clinical rating. Moreover, compensatory mechanisms may allow patients still to function, although marked damage has already occurred (29). In contrast, EPs are closely linked to the pathophysiology of disturbed signal conduction (7, 8, 14), regardless of whether delayed responses are clinically symptomatic or remain subclinical. The transformation of such subclinical pathology into clinical disability is the most likely explanation for the prognostic power of multimodal EP assessment [review in (9)].

The stimulation protocol used in the current and in previous studies of our group (11, 30) differs from the recommendations

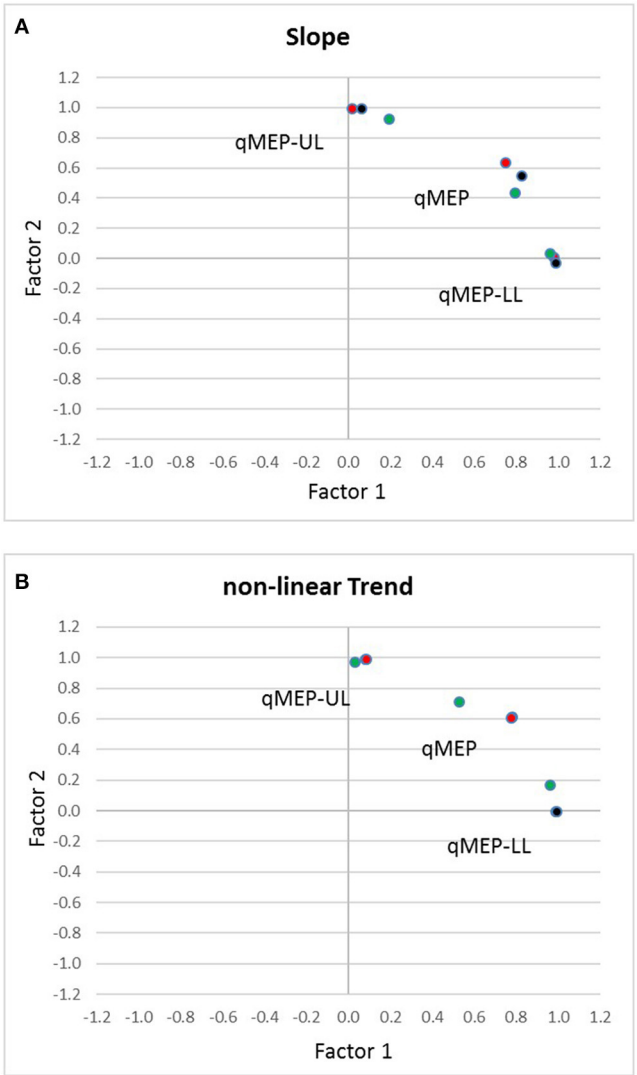


FIGURE 2 | Diagrams show the separation of quantitative MEP scores for upper (qMEP-UL) and lower limbs (qMEP-LL), as well as the combination of both (qMEP) along two independent dimensions obtained by factor analysis after Varimax rotation using **(A)** individual slopes (linear trends) **(B)** individual deviations from linearity (non-linear trends). The *x*- and *y*-coordinates of the variables are defined by their loadings on the first and second factors. Colors represent the different ways of calculating the scores from central motor conduction time (CMCT; red), shortest corticomuscular latency (CxM-sh; black), and mean CxM (CxM-mn; green).

of the IFCN regarding the determination of the resting motor threshold (RMT) (13). The standard method (31) is time consuming and requires the application of up to 75 stimuli. A proposed optimization of the method needs handling of additional software (32). The use of a standard stimulation intensity of 80 to 100% of stimulator output with a non-focal round coil is a pragmatic approach, which is time-efficient and easy to standardize. It induces a supramaximal cortical stimulation in nearly all subjects with a small overall number of stimuli. Moreover, it is probably near the recommended stimulation intensity of 140 to 170% RMT taking into account that RMT is higher in MS (33), and on average at 70% of stimulator output according to one study with progressive MS (34).

The main limitation of the current study is its small sample size, which greatly reduces the generalizability of the current findings. Furthermore, NHPT and T25FW were only available in a subgroup, rendering the comparison between these timed assessments and qMEP scores preliminary. However, our main results replicate the findings of a previous study in an independent sample (11), corroborating the validity of the use of EP for measuring change.

CONCLUSIONS

The current study confirms a finding of our previous study demonstrating that deterioration in a qEPS occurs earlier than clinical progression as measured by the EDSS in patients with primary progressive MS. Both upper and lower limb qMEP scores contribute independently to measuring change, and qMEP scores calculated from mean CxM showed slightly higher effect sizes than scores calculated from shortest CxM or CMCT. In most target populations, a combined qMEP score based on upper and lower limbs mean CxM is therefore a reasonable choice. The previously used multimodal qEPS may even increase the sensitivity to change.

The capability to detect subclinical change is a unique property of EP and complementary to clinical examination. Evoked potential assessment may even open a window within which therapeutic effects can be quantified, before a clinical effect is detectable. These features and the current results underline the role of EP as a candidate biomarker to measure effects of therapeutic interventions in PPMS.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available. The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by formerly Ethikkommission beider Basel, EKBB; currently Ethikkommission Nordwest- und Zentralschweiz, EKNZ; which approved the study protocols (EKBB 206/13; EKBB 161/12). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MH, CS, JK, and PF contributed to the study design, data acquisition, data analysis, data interpretation, manuscript drafting, and approved the final version. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The study has been financially supported by the Swiss Multiple Sclerosis Society and the research pool of

the Department of Neurology, University Hospital Basel. We would like to thank all participating patients, the clinical colleagues caring for the patients as well as the technical staff at the Division of Neurophysiology.

REFERENCES

1. Barkhof F, Calabresi PA, Miller DH, Reingold SC. Imaging outcomes for neuroprotection and repair in multiple sclerosis trials. *Nat Rev Neurol*. (2009) 5:256–66. doi: 10.1038/nrneurol.2009.41
2. Pardini M, Cutter G, Sormani MP. Multiple sclerosis: clinical trial design 2019. *Curr Opin Neurol*. (2019) 32:358–64. doi: 10.1097/WCO.0000000000000697
3. Moccia M, de Stefano N, Barkhof F. Imaging outcome measures for progressive multiple sclerosis trials. *Mult Scler*. (2017) 23:1614–26. doi: 10.1177/1352458517729456
4. Ontaneda D, Fox RJ. Imaging as an outcome measure in multiple sclerosis. *Neurotherapeutics*. (2017) 14:24–34. doi: 10.1007/s13311-016-0479-6
5. Barro C, Leocani L, Leppert D, Comi G, Kappos L, Kuhle J. Fluid biomarker and electrophysiological outcome measures for progressive MS trials. *Mult Scler*. (2017) 23:1600–13. doi: 10.1177/1352458517732844
6. McDonald WI, Sears TA. Effect of demyelination on conduction in the central nervous system. *Nature*. (1969) 221:182–3. doi: 10.1038/221182a0
7. Heidari M, Radcliff AB, McLellan GJ, Ver Hoeve JN, Chan K, Kiland JA, et al. Evoked potentials as a biomarker of remyelination. *Proc Natl Acad Sci USA*. (2019) 116:27074–83. doi: 10.1073/pnas.1906358116
8. Farley BJ, Morozova E, Dion J, Wang B, Harvey BD, Gianni D, et al. Evoked potentials as a translatable biomarker to track functional remyelination. *Mol Cell Neurosci*. (2019) 99:103393. doi: 10.1016/j.mcn.2019.103393
9. Hardmeier M, Leocani L, Fuhr P. A new role for evoked potentials in MS? Repurposing evoked potentials as biomarkers for clinical trials in MS. *Mult Scler*. (2017) 23:1309–19. doi: 10.1177/1352458517707265
10. Hardmeier M, Jacques F, Albrecht P, Bousleiman H, Schindler C, Leocani L, et al. Multicentre assessment of motor and sensory evoked potentials in multiple sclerosis: reliability and implications for clinical trials. *Mult Scler J Exp Transl Clin*. (2019) 5:2055217319844796. doi: 10.1177/2055217319844796
11. Schlaeger R, D'Souza M, Schindler C, Grize L, Kappos L, Fuhr P. Electrophysiological markers and predictors of the disease course in primary progressive multiple sclerosis. *Mult Scler*. (2014) 20:51–6. doi: 10.1177/1352458513490543
12. Comi G, Leocani L, Medaglini S, Locatelli T, Martinelli V, Santuccio G, et al. Measuring evoked responses in multiple sclerosis. *Mult Scler*. (1999) 5:263–7. doi: 10.1177/135245859900500412
13. Groppa S, Oliviero A, Eisen A, Quartarone A, Cohen LG, Mall V, et al. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol*. (2012) 123:858–82. doi: 10.1016/j.clinph.2012.01.010
14. Smith KJ. Conduction properties of central demyelinated and remyelinated axons, and their relation to symptom production in demyelinating disorders. *Eye*. (1994) 8:224–37. doi: 10.1038/eye.1994.51
15. Britton TC, Meyer BU, Benecke R. Variability of cortically evoked motor responses in multiple sclerosis. *Electroencephalogr Clin Neurophysiol*. (1991) 81:186–94. doi: 10.1016/0168-5597(91)90071-5
16. Kallmann BA, Fackelmann S, Toyka KV, Rieckmann P, Reiners K. Early abnormalities of evoked potentials and future disability in patients with multiple sclerosis. *Mult Scler*. (2006) 12:58–65. doi: 10.1191/135248506ms1244oa
17. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol*. (2018) 17:162–73. doi: 10.1016/S1474-4422(17)30470-2
18. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. (1983) 33:1444–52. doi: 10.1212/WNL.33.11.1444
19. Kappos L, D'Souza M, Lechner-Scott J, Lienert C. On the origin of neurostatus. *Mult Scler Relat Disord*. (2015) 4:182–5. doi: 10.1016/j.msard.2015.04.001
20. Fischer JS, Rudick RA, Cutter GR, Reingold SC. The Multiple Sclerosis Functional Composite Measure (MSFC): an integrated approach to MS clinical outcome assessment. *Mult Scler*. (1999) 5:244–50. doi: 10.1177/135245859900500409
21. Pisa M, Chieffo R, Giordano A, Gelibter S, Comola M, Comi G, et al. Upper limb motor evoked potentials as outcome measure in progressive multiple sclerosis. *Clin Neurophysiol*. (2020) 131:401–5. doi: 10.1016/j.clinph.2019.11.024
22. Di Lazzaro V, Ziemann U, Lemon RN. State of the art: physiology of transcranial motor cortex stimulation. *Brain Stimul*. (2008) 1:345–62. doi: 10.1016/j.brs.2008.07.004
23. Zhang J, Waubant E, Cutter G, Wolinsky J, Leppert D. Composite end points to assess delay of disability progression by MS treatments. *Mult Scler*. (2014) 20:1494–501. doi: 10.1177/1352458514527180
24. Cadavid D, Cohen JA, Freedman MS, Goldman MD, Hartung HP, Havrdova E, et al. The EDSS-Plus, an improved endpoint for disability progression in secondary progressive multiple sclerosis. *Mult Scler*. (2017) 23:94–105. doi: 10.1177/1352458516638941
25. Zeller D, Reiners K, Bräuninger S, Buttmann M. Central motor conduction time may predict response to fampridine in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry*. (2014) 85:707–9. doi: 10.1136/jnnp-2013-306860
26. Capone F, Capone G, Motolese F, Voci A, Caminiti ML, Musumeci G, et al. Spinal cord dysfunction contributes to balance impairment in multiple sclerosis patients. *Clin Neurol Neurosurg*. (2019) 184:105451. doi: 10.1016/j.clineuro.2019.105451
27. Feys P, Lamers I, Francis G, Benedict R, Phillips G, LaRocca N, et al. The Nine-Hole Peg Test as a manual dexterity performance measure for multiple sclerosis. *Mult Scler*. (2017) 23:711–20. doi: 10.1177/1352458517690824
28. Motl RW, Cohen JA, Benedict R, Phillips G, LaRocca N, Hudson LD, et al. Validity of the timed 25-foot walk as an ambulatory performance outcome measure for multiple sclerosis. *Mult Scler*. (2017) 23:704–10. doi: 10.1177/1352458517690823
29. Schoonheim MM, Geurts JJ, Barkhof F. The limits of functional reorganization in multiple sclerosis. *Neurology*. (2010) 74:1246–7. doi: 10.1212/WNL.0b013e3181db9957
30. Fuhr P, Borggrefe-Chappuis A, Schindler C, Kappos L. Visual and motor evoked potentials in the course of multiple sclerosis. *Brain*. (2001) 124:2162–8. doi: 10.1093/brain/124.11.2162
31. Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol*. (1994) 91:79–92. doi: 10.1016/0013-4694(94)90029-9
32. Awiszus F. Fast estimation of transcranial magnetic stimulation motor threshold: is it safe? *Brain Stimul*. (2011) 4:58–9. doi: 10.1016/j.brs.2010.09.004
33. Caramia MD, Palmieri MG, Desiato MT, Boffa L, Galizia P, Rossini PM, et al. Brain excitability changes in the relapsing and remitting phases of multiple sclerosis: a study with transcranial magnetic stimulation. *Clin Neurophysiol*. (2004) 115:956–65. doi: 10.1016/j.clinph.2003.11.024
34. Ayache SS, Créange A, Farhat WH, Zouari HG, Lesage C, Palm U, et al. Cortical excitability changes over time in progressive multiple sclerosis. *Funct Neurol*. (2015) 30:257–63. doi: 10.11138/FNeur/2015.30.4.257

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00735/full#supplementary-material

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

frontiers in Neurology

ORIGINAL RESEARCH
published: 15 October 2020
doi: 10.3389/fneur.2020.545377

Effects of Transcranial Direct Current Stimulation on Information Processing Speed, Working Memory, Attention, and Social Cognition in Multiple Sclerosis

Christina Grigorescu[1†], *Moussa A. Chalah*[2,3†], *Jean-Pascal Lefaucheur*[2,3], *Tania Kümpfel*[4], *Frank Padberg*[1], *Samar S. Ayache*[2,3†] *and Ulrich Palm*[1,5*†]

[1] Department of Psychiatry and Psychotherapy, Klinikum der Universität München, Munich, Germany, [2] EA 4391, Excitabilité nerveuse et thérapeutique, Université Paris-Est-Créteil, Créteil, France, [3] Service de Physiologie-Explorations Fonctionnelles, Hôpital Henri-Mondor, Assistance Publique-Hôpitaux de Paris, Créteil, France, [4] Institute for Clinical Neuroimmunology, Klinikum der Universität München, Munich, Germany, [5] Medical Park Chiemseeblick, Bernau, Germany

OPEN ACCESS

Edited by:
Sharon Glynn Lynch,
University of Kansas Medical Center,
United States

Reviewed by:
Jordi A. Matias-Guiu,
Hospital Clínico San Carlos, Spain
Alberto Benussi,
University of Brescia, Italy

***Correspondence:**
Ulrich Palm
u.palm@medicalpark.de

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: 24 March 2020
Accepted: 26 August 2020
Published: 15 October 2020

Citation:
Grigorescu C, Chalah MA, Lefaucheur J-P, Kümpfel T, Padberg F, Ayache SS and Palm U (2020) Effects of Transcranial Direct Current Stimulation on Information Processing Speed, Working Memory, Attention, and Social Cognition in Multiple Sclerosis. Front. Neurol. 11:545377. doi: 10.3389/fneur.2020.545377

Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system. Cognitive impairment occurs in 40–65% of patients and could drastically affect their quality of life. Deficits could involve general cognition (e.g., attention and working memory) as well as social cognition. Transcranial direct current stimulation (tDCS), is a novel brain stimulation technique that has been assessed in the context of several neuropsychiatric symptoms, including those described in the context of MS. However, very rare trials have assessed tDCS effects on general cognition in MS, and none has tackled social cognition. The aim of this work was to assess tDCS effects on general and social cognition in MS. Eleven right-handed patients with MS received two blocks (bifrontal tDCS and sham, 2 mA, 20 min, anode/cathode over left/right prefrontal cortex) of 5 daily stimulations separated by a 3-week washout interval. Working memory and attention were, respectively, measured using N-Back Test (0-Back, 1-Back, and 2-Back) and Symbol Digit Modalities Test (SDMT) at the first and fifth day of each block and 1 week later. Social cognition was evaluated using Faux Pas Test and Eyes Test at baseline and 1 week after each block. Interestingly, accuracy of 1-Back test improved following sham but not active bifrontal tDCS. Therefore, active bifrontal tDCS could have impaired working memory via cathodal stimulation of the right prefrontal cortex. No significant tDCS effects were observed on social cognitive measures and SDMT. Admitting the small sample size and the learning (practice) effect that might arise from the repetitive administration of each task, the current results should be considered as preliminary and further investigations in larger patient samples are needed to gain a closer understanding of tDCS effects on cognition in MS.

Keywords: tDCS, social cognition, theory of mind, faux pas test, N-back test, attention, working memory, information processing speed

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system, characterized by demyelination, neurodegeneration and synaptopathy (1, 2). It occurs in around 2.3–2.5 million around the globe and is the most common reason of non-traumatic disability in young people (3). Patients with MS (PwMS) could suffer from several symptoms including sensorimotor deficits, cerebellar symptoms, fatigue, as well as emotional, cognitive, and behavioral manifestations (4). Cognitive impairment occurs in 40–65% of patients at one point in their lifetime, could appear in early stages of MS (5, 6) and has a drastic impact on patients' quality of life as well as their daily activities. Cognitive impairment could affect general cognition, such as learning and memory, attention (i.e., information processing speed (IPS), complex, divided and selection attention), language, perceptual-motor and executive functions, as well as social cognition (7–10).

Memory and IPS are among the most deficient cognitive domains in MS (11). Memory impairment occurs in up to 40–65% of patients, with working and long-term memory being importantly affected in the context of MS (12, 13). IPS deficit could be observed in 20–30% of PwMS (9, 13, 14). It is related to decreased neuronal conduction speed secondary to demyelination, and can halt the individual's ability to complete tasks and cope with demanding everyday life requirements (13, 15).

Besides general cognition, there was a recent growing interest to assess the involvement of social cognition in the process of MS [for reviews see (10)]. Social cognition can be seen as mental operations put into action during social interactions, including perception, interpretation, and generating responses to the intentions, dispositions, and behaviors of others (16). Social cognition entails the individual's ability to (a) recognize emotions from social stimuli cues, (b) infer others' mental state based on their intentions, thoughts and beliefs [i.e., cognitive theory of mind (ToM)], and their emotions and desires (i.e., affective ToM), and (c) empathize with others (10, 17, 18). Social cognition influences the relationship with friends, family, colleagues, and strangers. Thus, it has a high impact on peer support which is a relevant factor for good quality of life and coping with everyday life difficulties, a coping that is particularly important in patients suffering from a chronic and debilitating disease such as MS (19). There is evidence that PwMS show considerable social cognitive deficits that are at the origin of additional burden in this population (10, 20, 21).

From a neurobiological perspective, neuroimaging studies have explored the structural and functional correlates of cognitive impairment in MS. Some studies linked cognitive impairment to pathologies involving the frontal, parietal, temporal and thalamic regions [For reviews see (22)]. It is noteworthy that the frontal lobe constitutes a carrefour for several cognitive tracts, and many studies have linked cognitive impairment in MS with abnormalities involving the (pre)-frontal cortex and/or its connectivity [For reviews see (23)].

From a therapeutic perspective, despite the serious impact of cognitive deficits on this population, efficacy of pharmacological and cognitive interventions has not been supported by enough evidence [For reviews see (11)]. Therefore, alternative interventions might have their place in this context, and their effects merit to be explored. Recently, non-invasive brain stimulation (NIBS), notably transcranial direct current stimulation (tDCS), has shown promising results in the treatment of MS-related symptoms, with most of studies focusing on MS fatigue (4, 24). However, tDCS effects on MS-related cognitive deficits have been rarely addressed. Positive effects have been suggested by few trials that have combined this technique with cognitive training (25, 26), or by some case reports [(10, 27–29), for a review see: 4].

The present report is part of a randomized double-blind sham-controlled cross-over study designed to assess the effects of anodal bifrontal tDCS on MS fatigue as well as other components of the symptoms cluster (i.e., anxiety and depression) (30). Five consecutive daily tDCS sessions led to acute antifatigue effects and delayed anxiolytic effects that emerged 1 week later (30).

Here, we aimed to study the effect of anodal bifrontal tDCS on general cognition (i.e., attention, working memory and IPS) and social cognition in PwMS. Neuroimaging studies (functional MRI and [^{11}C]-raclopride Positron emission tomography performed in healthy individuals) and computational model analysis suggest that bifrontal tDCS could modulate the function of cortico-subcortical circuits [i.e., (31–33)]. In MS, despite the lack of studies assessing tDCS mechanisms of action on cognitive functions, the application of high frequency repetitive transcranial magnetic stimulation (another NIBS technique which is supposed to activate the cortical area in question) over the left prefrontal cortex resulted in a cognitive improvement that was paralleled by an increase in prefrontal functional connectivity (34). Therefore, following the same logic, we hypothesized that enhancing the activity of frontal regions and their connectivity, by applying tDCS, could improve general and social cognitive performance in this clinical population.

MATERIALS AND METHODS

Participants

The study took place at the Department of Psychiatry and Psychotherapy of University of Munich Hospital. Recruitment occurred at the Institute of Clinical Neuroimmunology and Cooperating Neurological Practices. Right-handed patients (age: 18–75 years), with a definite MS diagnosis [according to 2017 revised McDonald criteria; (35)] and low disability [Expanded Disability Status Scale score (EDSS) < 6.5; (36)] took part of the study. They had stable treatments ($\geq$ 1 month) and did not suffer from relapses (During the last 2 months), or other relevant neuropsychiatric diseases [inclusion/exclusion criteria details are reported in Chalah et al. (30)]. The local ethics committee approved the study which was conducted in conformity with the declaration of Helsinki. All patients gave written informed consent prior to inclusion. Eleven patients (8 females) participated in the study protocol.

Evaluation

Attention, Working Memory, and Information Processing Speed

The N-Back task is commonly used to assess working memory in MS studies (37, 38). In addition, this task has been widely adopted in tDCS studies that documented working memory improvement in healthy and some neuropsychiatric populations [for review and meta-analysis, please refer to Brunoni and Vanderhasselt (39)]. Among these studies, some have documented improvement in N-Back outcomes following the application of a single session of bifrontal tDCS (anode/cathode over F3/F4) in healthy individuals [$n = 10$, (40)] or depressed patients [$n = 28$, (41)].

N-Back v5 was used in this study. Presentation of visual stimuli and recording of responses were controlled using Presentation Software (Neurobehavioral Systems, Inc., Albany, CA, USA). In this experiment, working memory was evaluated using three difficulty levels, the latter differ in the number of items to memorize (i.e., 0, 1, or 2 items) and refer to as 0-Back, 1-Back, and 2-Back. The 0-Back condition is the easiest one in which the target consisted of any item that matches a pre-specified item, and hence this condition requires sustained attention but no working memory demand (42). The two other conditions are of increasing difficulties and evaluate working memory. The targets of the 1-Back and 2-Back conditions correspond to any item identical to the item presented one trial and two trials back, respectively. For each condition, results are displayed as accuracy and reaction time.

Symbol Digit Modalities Test (SDMT) was used to assess IPS and visuospatial attention (43). This task was adopted because it is easy to use, fast to administer, does not cause any significant amount of stress for patients, and is considered a sentinel test to assess cognitive status in PwMS (44). In addition, this test was previously employed in the only two available studies assessing tDCS effects on cognition in PwMS (25, 26). A key that pairs single digits with nine symbols is presented, and the individual is asked to fill rows containing only symbols by matching them with the correct numbers according to the key. The score corresponds to the total number of correct answers that the individual obtains in 90 s. The same versions of SDMT and N-Back were used during all the evaluations.

Social Cognition

Social cognition was assessed by means of Reading the Mind in the Eyes Test (Eyes Test) and Faux Pas Test, which, respectively, assess the affective and cognitive components of ToM (45–47). Eyes Test is a non-verbal test that assesses the affective component of ToM and consists of 36 eye pictures of actors and actresses expressing several emotional states and the patient is asked to interpret the social sign hidden in the pair of eyes. Initially developed for autism disorders, other psychiatric and neurological patients were found to poorly perform on this test (10, 45, 47, 48). Eyes Test score is calculated by summing up all individual items, with higher scores indicating better skills.

Faux Pas Test is a verbal test that measures cognitive ToM (46). The test assesses the ability of an individual to detect a "faux pas" which could occur "when a speaker says something without considering if it is something that the listener might not want to hear or know, and which typically has negative consequences that the speaker never intended" (46). The test consists of reading faux pas stories and control stories for individuals. Afterwards, the individual is assessed for their capacity to understand inappropriateness, intentions, and false beliefs. For the Faux Pas Test, individual scores given for short stories are summed up. The higher the score the better the performance is. The same versions of Eyes Test and Faux Pas Test were used during all the evaluations.

Transcranial Direct Current Stimulation

A weak electric current (i.e., 2 mA) is applied via a CE-certified battery driven stimulator (Eldith DC stimulator, NeuroConn, Ilmenau, Germany) and two saline-soaked sponge electrodes (5 cm x 7 cm) fixed by rubber bands with the anode and cathode over the left and right dorsolateral prefrontal cortices (DLPFC) (F3 and F4 according to the EEG 10–20 system) (49). tDCS setup is presented in **Figure 1A**. Patients were randomly assigned to receive active and sham tDCS blocks in a cross-over design. Each block consisted of five consecutive daily sessions, with 20 min per session (**Figure 1B**). Blocks were separated by a 3-week washout interval. For the active condition, current ramping up and down was done over 15 s at the beginning and end of each session, respectively, separated by 20 min stimulation. For sham, the same pattern of ramping was performed with only 30 s of stimulation aiming to simulate the cutaneous sensations obtained with active tDCS (50). tDCS parameters (i.e., current intensity, polarity, sessions number and duration) were chosen according to previous works performed in MS and other clinical populations [for reviews see (4, 24)].

Study Protocol

Patients were evaluated for eligibility. In case of eligibility and agreement to participate in the study, patients gave their informed written consent, underwent a baseline evaluation (T0), and were randomized to receive tDCS blocks. Allocation to start with an active or sham treatment was performed by a computerized random generator.

In each block, tDCS was applied from the first day (T1) to the 5th day (T2) while patients were at rest, sitting in a comfortable chair in a quiet room.

N-Back Test and SDMT were performed at T1 and T2, and 1 week after each block (T3). Given the potential susceptibility of social cognitive measures to practice effects (51), as well as the absence of learning effect and the acceptable test-retest reliability that are reported when repeating these measures few weeks after a first evaluation [i.e., (52, 53)], Eyes Test and Faux Pas Test were assessed at T0 and T3 of each block. **Figure 1C** provides a schematic presentation of the study design.

Statistical Analysis

Statistical analyses were performed using SPSS software (Version 24.0, Armonk, NY: IBM Corp.) and all measures were compared between active and sham conditions. Since not all data followed normal distribution according to Kolmogorov-Smirnov test, a non-parametric analysis of variance was run for group

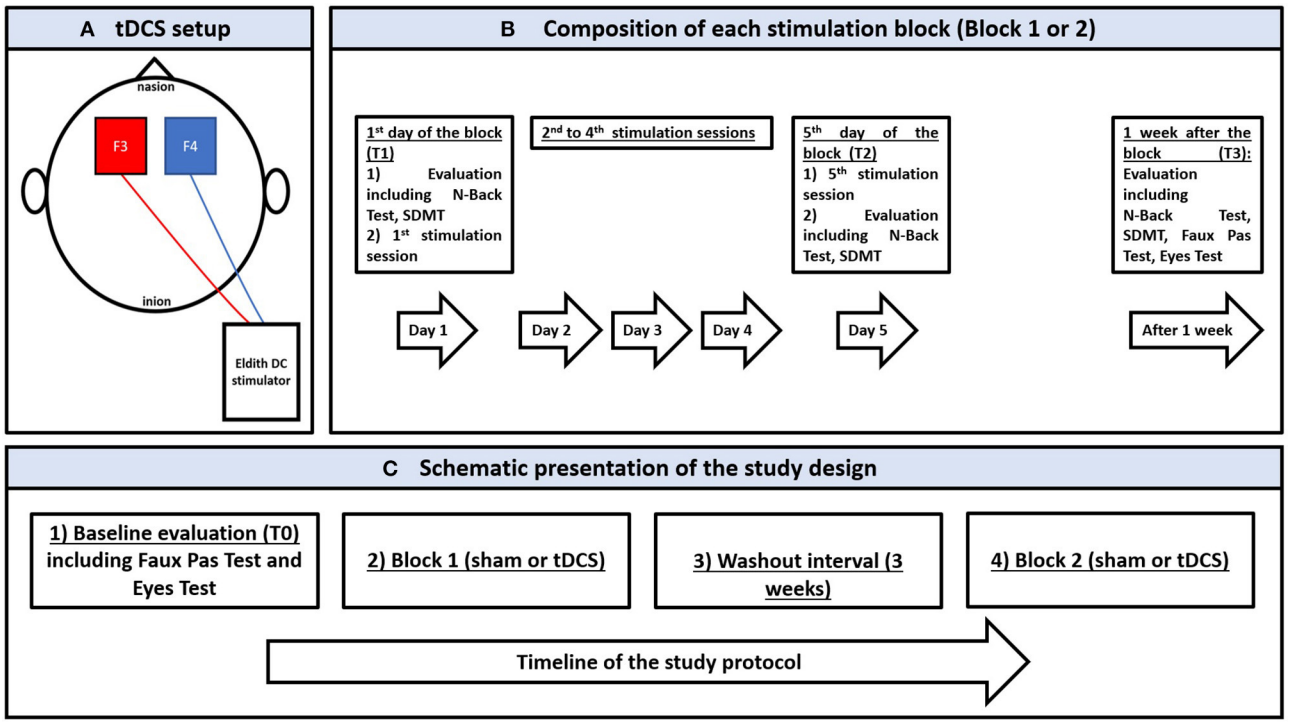


FIGURE 1 | (A) schematic presentation of the transcranial direct stimulation setup showing the anode (in red) and the cathode (in blue) over F3 and F4, respectively, according to the 10–20 international electroencephalographic system for electrode positioning; the electrodes are connected to a battery-driver direct current stimulator. **(B)** schematic presentation of a stimulation block (sham or active) showing the stimulation and evaluation sessions between the 1st day of stimulation and 1 week after the last stimulation. **(C)** schematic presentation of the study protocol showing the chronological order of experimentation. SDMT: Symbol Digit Modalities Test.

comparison using Friedman's test and *post-hoc* Dunn's test, and took into consideration the groups (active vs. sham) and the time points (T1, T2, and T3 for N-back Test and SDMT; T0 and T3 in the case of Eyes Test and Faux Pas Test). For Friedman's test, estimation of effect size was based on Kendall's W coefficient of concordance (54), with effect size being considered small (<0.3), moderate (0.3–0.5) and large ($\geq$ 0.5). To test for a possible carry-over effect (i.e., effects from the first block that could persist in the second block), Wilcoxon's test was run on data obtained prior to each stimulation block (pre-active vs. pre-sham). In addition, to test for possible learning that could result from repeated exposure to the same tests (i.e., practice effect), the patients' scores on each test were grouped according to the chronological order of evaluations (regardless which stimulation condition was administered first), and were compared using Friedman's test and *post-hoc* Dunn's test. For all tests, significance was set at 0.05. Data are presented as mean $\pm$ SD.

RESULTS

Sociodemographic and Clinical Data

The mean patients' age was 43.91 $\pm$ 9.69 years (age range 26–57 years). Mean disease duration was 75.64 $\pm$ 45.97 months. Mean EDSS was 3.14 $\pm$ 1.31. Ten patients had a relapsing-remitting MS and 1 patient had a secondary-progressive MS. Nine patients were receiving immunomodulatory treatments. tDCS was well-tolerated and there were no serious adverse effects at any time. tDCS safety and patients' clinical discomfort did not significantly differ according to the stimulation condition (30).

Cognitive Data

No significant differences were observed in cognitive scores obtained prior to the active and sham interventions (Wilcoxon's test).

Attention, Working Memory and Information Processing Speed

Concerning the outcomes obtained with N-Back test, Friedman's test of differences among repeated measures rendered a X^2 value of 13.14 which was only significant in the case of 1-Back accuracy ($df = 5$; $p = 0.022$). *Post-hoc* analysis revealed significant effects obtained, not right after sham intervention (T1: 0.83 $\pm$ 0.16 vs. T2: 0.76 $\pm$ 0.38; p>0.05), but rather 1 week later (T3: 0.94 $\pm$ 0.09, $p < 0.05$) (**Figure 2**). Effects of active intervention did not reach statistical significance right after the intervention (T1: 0.91 $\pm$ 0.10 vs. T2: 0.99 $\pm$ 0.03; $p > 0.05$) or 1 week later (T3: 0.78 $\pm$ 0.31, $p > 0.05$) (**Table 1**). 1-Back accuracy at T1 did not differ between active and sham conditions (p>0.05). Results of N-Back outcomes are summarized in **Table 1**.

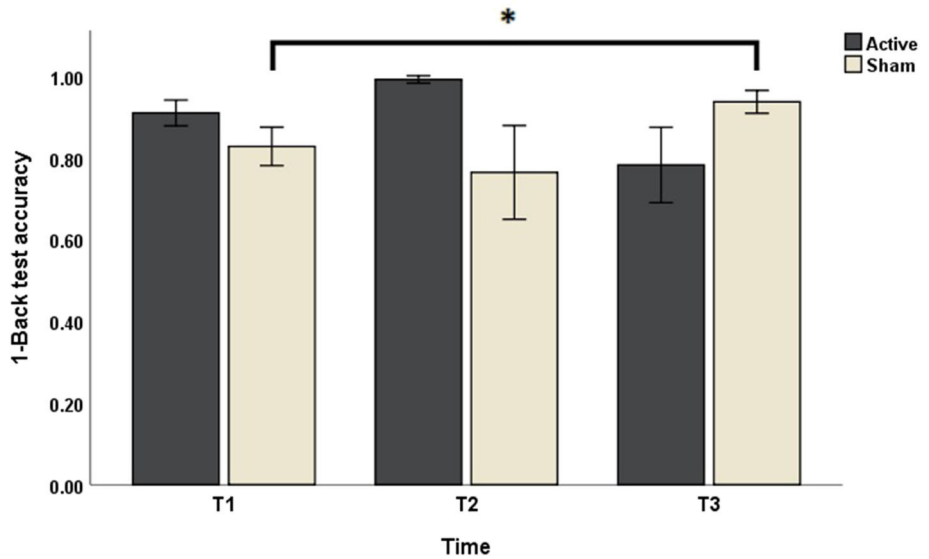


FIGURE 2 | The effects of sham and active stimulation on 1-Back test accuracy. T1, T2, and T3: day 1, day 5, and 1 week after each stimulation block, respectively. *$p < 0.05$.

Concerning SDMT scores, Friedman's test rendered a X^2 value of 10.48 which was non-significant ($df = 5$; $p = 0.063$) (**Table 1**). Kendall's W coefficient of concordance was < 0.3 (small effect size) for all outcomes (details are mentioned in **Table 1**).

When studying the learning effect, a significant learning effect was observed regarding SDMT scores (Friedman's test $p < 0.001$). *Post-hoc* analysis revealed significant increases in SDMT scores starting the 4th evaluation ($p < 0.05$).

No significant learning effect was observed regarding 0-Back accuracy (Friedman's Test $p = 0.069$), 0-Back reaction time (Friedman's Test $p = 0.391$), 1-Back accuracy (Friedman's Test p=0.191), 1-Back reaction time (Friedman's Test p=0.582), 2-Back accuracy (Friedman's Test $p = 0.169$) or 2-Back reaction time (Friedman's Test $p = 0.652$).

Social Cognition

Friedman's test showed a X^2 value of 1.81 for the Eyes Test which was non-significant ($df = 2$, $p = 0.406$). The same applies to the Faux Pas Test where no significant difference was observed following sham and active conditions (Friedman's Test X^2= 2.61; $df = 2$; $p = 0.272$). **Table 2** presents the different scores obtained at each time point. Kendall's W coefficient of concordance was < 0.3 (small effect size) for both tests (details are mentioned in **Table 1**). No significant learning effect was observed for the Eyes Test (Friedman's Test $p = 0.803$) or Faux Pas Test (Friedman's Test $p = 0.307$).

DISCUSSION

This study evaluated tDCS effects on general cognition (particularly attention, working memory, and IPS), as assessed by N-Back Test and SDMT, and social cognition, according to Faux Pas and Eyes Tests, in patients with MS. The main finding of this work consisted of a significant delayed improvement in 1-Back accuracy obtained 1 week after sham intervention. This outcome was not found with active stimulation. Neither intervention had significant effects on the remaining outcomes.

tDCS and Attention, Working Memory, and Information Processing Speed

Cognitive performance (accuracy in 1-Back Test) interestingly improved after sham, but not after tDCS condition. A systematic review reports mixed effects of anodal tDCS on working memory performance (55). Our results are in line with previous findings on this matter (56–58). For instance, in studies involving healthy participants, anodal bifrontal tDCS hampered the accuracy (58). In addition, in a study involving patients with major depressive disorder, the accuracy on procedural or implicit learning task improved following sham but not active stimulation as seen in our present work (57). The authors concluded that bifrontal tDCS prevented implicit learning in their cohort.

Here, it is worth noting that the negative findings of the current study could be partly attributed to the low statistical power of our sample, although in some studies using similar sample size ($n = 10$), a single 10 min session of bifrontal tDCS was able to improve N-Back outcomes in healthy volunteers (40). In this context, it is important to mention that tDCS response might differ between the healthy and diseased brain, as well as across clinical populations. In fact, Hill and colleagues have reported a significant improvement in offline working memory tasks in healthy but not in neuropsychiatric cohorts (55). Relative to healthy individuals, MS patients might suffer from baseline cortico-subcortical abnormalities and changes in regional connectivity, which might have compromised the emergence of robust changes.

TABLE 1 | The effects of sham and active stimulation on N-Back Test (0-Back, 1-Back and 2-Back) and Symbol Digit Modalities Test (SDMT).

Studied outcomes	Friedman's test *p*-value	Kendall's *W*	Active stimulation				Sham stimulation				Dunn's test *p*-value
			T1	T2	T3	Dunn's test *p*-value	T1	T2	T3	Dunn's test *p*-value	
0-Back accuracy	0.157	0.145	0.84 ± 0.35	0.90 ± 0.30	–	>0.05	0.77 ± 0.39	0.99 ± 0.03	–	>0.05	
				–	0.99 ± 0.03	>0.05		–	0.98 ± 0.06	>0.05	
0-Back RT (ms)	**0.044**	0.207	541.47 ± 200.68	498.04 ± 186.69	–	>0.05	445.64 ± 244.80	514.76 ± 81.84	–	>0.05	
			541.47 ± 200.68	–	558.30 ± 85.34	>0.05	445.64 ± 244.80	–	514.15 ± 76.62	>0.05	
1-Back accuracy	**0.022**	0.239	0.91 ± 0.10	0.99 ± 0.03	–	>0.05	0.83 ± 0.16	0.76 ± 0.38	–	>0.05	
			0.91 ± 0.10	–	0.78 ± 0.31	>0.05	0.83 ± 0.16	–	0.94 ± 0.09	**<0.05**	
1-Back RT (ms)	0.628	0.063	625.84 ± 106.43	600.86 ± 98.38	–	>0.05	633.72 ± 114.93	599.26 ± 222.83	–	>0.05	
			625.84 ± 106.43	–	570.47 ± 217.95	>0.05	633.72 ± 114.93	–	567.58 ± 62.49	>0.05	
2-Back accuracy	0.235	0.124	0.68 ± 0.15	0.74 ± 0.24	–	>0.05	0.68 ± 0.17	0.67 ± 0.28	–	>0.05	
			0.68 ± 0.15	–	0.78 ± 0.16	0.05	0.68 ± 0.17	–	0.77 ±0.12	>0.05	
2-Back RT (ms)	0.732	0.051	717.59 ± 93.62	741.46 ± 154.31	–	>0.05	754.33 ± 174.91	808.40 ± 193.70	–	>0.05	
			717.59 ± 93.62	–	708.07 ± 144.24	>0.05	754.33 ± 174.91	–	696.64 ± 110.46	>0.05	
SDMT	0.063	0.191	49.73 ± 12.28	54.00 ± 14.07	–	>0.05	48.55 ± 10.89	50.45 ± 10.98	–	>0.05	
			49.73 ± 12.28	–	55.64 ± 11.63	>0.05	48.55 ± 10.89	–	52.73 ± 9.47	>0.05	

T1, T2, and T3: day 1, day 5, and 1 week after each stimulation block, respectively. RT, reaction time. Significant p-values are bolded.

Another possible explanation of our negative findings could be the placement of the reference electrodes. In fact, in bifrontal montage, the reference electrode is over the right DLPFC. It seems that this have resulted in cathodal stimulation of this area, and hence an inhibition of cognitive processes to which it contributes. Here, it is worth noting that the right DLPFC is an important carrefour that gets activated during visual working memory tasks (59–62), and a damage of this area could impair working memory as demonstrated in lesion studies (61, 63, 64). Therefore, in our work, the relative improvement of working memory obtained following sham intervention would indirectly hint toward an impairment of this cognitive ability following active condition. An impairment that is probably due to the inhibition of right DLPFC by cathodal stimulation.

Several works have highlighted the role of the right DLPFC in working memory (65–67), among which some consisted of tDCS works that documented an improvement of working memory when placing the anode over this area in healthy populations [cathode: over left cheek in Wu et al. (68); over the contralateral supraorbital area in Giglia et al. (69); over Cz in Bogdanov and Schwabe (70); and over F3 in Nissim et al. (33)]. Therefore, it would be interesting in future works to set the anode over this area (F4) and determine the optimal return electrode to ameliorate working memory.

Besides working memory, attention and IPS do not seem to be affected by tDCS in this study, although the observed learning effect might have prohibited observing such changes. The current findings are consistent with previous works that reported lack of tDCS effects on attention or IPS when applied over 3–5 consecutive days in MS (10, 71, 72). Conversely, few trials suggested the add-on benefits of 10 sessions of anodal prefontal tDCS stimulation when combined with cognitive training (25, 26). Moreover, positive effects were reported in few case reports that applied 14–40 anodal tDCS sessions over the left prefrontal cortex (10, 27, 28). Therefore, longer stimulation duration and combination with cognitive training might improve cognition.

tDCS and Social Cognition

Social cognition did not significantly improve following tDCS. To the best of our knowledge, this is the first study to address the effects of tDCS on social cognition in MS. The idea of modulating social cognition by tDCS targeting the prefrontal cortex stems from studies showing the involvement of this region in social cognition (73), and its relationship with social cognitive deficits in MS (10). Unlike our study, 12 sessions of bifrontal tDCS improved social cognition in depressed patients (74). Moreover, a single session of anodal tDCS ameliorated social cognitive measures in healthy individuals [left prefrontal anodal stimulation, right frontopolar cathode; (75)], as in patients with neurodegenerative diseases [medial prefrontal anodal stimulation; in frontotemporal dementia (76) and in Parkinson's disease (77)]. It is noteworthy that, with regards to social cognition, a hemispheric asymmetry seems to exist for some processes, and the right cerebral hemisphere appears to be important for social cognitive processes (78, 79). Therefore, as suggested for working memory, it would be of interest when targeting social cognition to test the application of anodal tDCS

TABLE 2 | The effects of sham and active stimulation on Eyes Test and Faux Pas Test scores.

Studied outcomes	Friedman's test *p*-value	Kendall's *W*	Time points			
			Baseline	Post-active stimulation	Post-sham stimulation	Dunn's test *p*-value
Eyes test	0.406	0.082	24.09 ± 4.97	23.27 ± 3.64	–	>0.05
			24.09 ± 4.97	–	24.73 ± 4.52	>0.05
Faux pas test	0.272	0.118	22.18 ± 3.71	20.55 ± 3.91	–	>0.05
			22.18 ± 3.71	–	19.45 ± 4.01	>0.05

All differences were not significant at 0.05.

over the right DLPFC. However, an attention should be paid when selecting the other electrode (the reference electrode) since an anodal F4/cathodal F3 setup was found in few works to negatively affect some social cognitive aspects such as adopting others' perspective (80) or empathy for pain (81).

Future studies would also benefit from increasing the number of stimulation sessions and investigating the utility of targeting other cortical areas, such as the right temporoparietal junction and the ventromedial prefrontal cortex (82–85).

Limitations and Perspectives

This study has several limitations. First, admitting the small sample size and the small effect size estimates (all below <0.3), this work should be considered a pilot study with non-definite preliminary results. Larger studies are needed to further explore these findings. Second, the cross-over design could have provoked overlapping effects. The wash-out interval of 3 weeks may be too short to prevent the effects from the first block to interfere with the second stimulation block.

Third, a limitation might arise from the employed tools to evaluate cognition. Although this study included cognitive measures that are widely used in MS research, the use of the same tests several times across the study is a key point to consider since it may imply a potential practice effect (86, 87), as was observed with SDMT scores in this work. Future studies would benefit from employing alternate forms of the cognitive tasks at each evaluation. In that context, alternate forms of SDMT have been proposed in MS studies; they are reliable and equivalent in difficulty which could help overcoming the practice effect when considering cognitive outcomes in future tDCS trials (88, 89). Similarly, alternate forms of memory tests (including N-Back test) using the same set of stimuli with different order or composition have also been suggested (90, 91). Moreover, retesting in social cognition may be problematic. Although the tests employed in this work stand among the most adopted in the literature (i.e., Eyes Test and Faux Pas Test), no alternate forms seem to exist for these tests. Thus, employing social cognitive tasks that are available in multiple forms (e.g., The Assessment of Social Inference Test, the Hinting Task) could help avoiding the practice effect [for reviews see (92)]. However, when choosing to employ the same vs. alternate forms of a task, it is also important to consider the possibility of statistically accounting for the practice effect related to the repeated administration of the same task as well as the challenges related to the use of alternate forms, namely the number of required forms that increases with the number of testing points and the differences in task difficulty across the different forms (87).

Fourth, although the evaluation of IPS and sustained attention included tasks that are considered simple, insight from neuroimaging studies suggest that some of these tasks are complex and recruit cortico-subcortical networks [(93–95). Therefore, including a simpler task might have been more sensitive to detect subtle tDCS effects; this could have been done using a simple reaction time task which for example requires the individual to press a button as soon as a single stimulus appears in the center of a computer screen (96). Besides the tasks' complexity, another drawback is related to the choice of social cognition tools. Social cognition is a complex construct of multiple components that was assessed by static tasks. Dynamic tasks (i.e., videotapes featuring social scenes) might have better ecological validity (10), and merits to be adopted in tDCS studies on MS. Future studies could also benefit from assessing tDCS effects on other general (i.e., perceptual-motor and executive functions, language) or social cognitive domains (i.e., emotion recognition from facial, vocal or bodily cues, empathic ability).

Finally, as stated above, it would be interesting to test different tDCS variables (i.e., polarity, electrode locations and montage, sessions number and duration, current intensity) in order to determine the optimal parameters to improve cognitive functions in MS. For instance, applying greater tDCS doses (i.e., intensity and duration) and/or combining them with other interventions might lead to synergistic effects. However, repeating the sessions and including patients in a protocol lasting several weeks might be difficult; a home-based and remotely supervised treatment could fill this gap (97).

CONCLUSIONS

This study assessed the effects of five consecutive daily 20 min sessions of bifrontal tDCS on cognition in MS. 1-Back accuracy improved after sham but not after active tDCS. Bifrontal tDCS seems to impair working memory in PwMS. No other significant effects were observed on attention, IPS, or social cognition. A larger patient sample and potentially a longer stimulation interval and follow up could help confirming the current results.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available to any qualified researcher on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ludwig Maximilian University Munich. The patients provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MAC, SSA, J-PL, FP, and UP designed the study. Participants were recruited by TK and screened by CG. CG performed data collection. MAC and SSA performed the analysis. The manuscript was drafted by CG, MAC, SSA, and UP. All authors participated to data interpretation, critically revised the manuscript, and approved the final version.

ACKNOWLEDGMENTS

This work is part of the Medical Doctor Thesis of CG.

REFERENCES

1. Compston A, Coles A. Multiple sclerosis. *Lancet*. (2018) 372:1502–17. doi: 10.1016/S0140-6736(08)61620-7
2. Chalah MA, Ayache SS. Is there a link between inflammation and fatigue in multiple sclerosis? *J Inflamm Res*. (2018) 11:253–64. doi: 10.2147/JIR.S167199
3. Hoffmann S, Vitzthum K, Mache S, Spallek M, Quarcoo D, Groneberg DA, et al. Multiple sklerose: epidemiologie, pathophysiologie, diagnostik und therapie. *Praktische Arbeitsmedizin*. (2009) 17:12–8.
4. Ayache SS, Chalah MA. The place of transcranial direct current stimulation in the management of multiple sclerosis-related symptoms. *Neurodegener Dis Manag*. (2018) 8:411–22. doi: 10.2217/nmt-2018-0028
5. Amato MP, Zipoli V, Portaccio E. Multiple sclerosis-related cognitive changes: a review of cross-sectional and longitudinal studies. *J Neurol Sci*. (2006) 245:41–6. doi: 10.1016/j.jns.2005.08.019
6. Kraemer M, Herold M, Uekermann J, Kis B, Wiltfang J, Daum I, et al. Theory of mind and empathy in patients at an early stage of relapsing remitting multiple sclerosis. *Clin Neurol Neurosurg*. (2013) 115:1016–22. doi: 10.1016/j.clineuro.2012.10.027
7. Benedict RH, Cookfair D, Gavett R, Gunther M, Munschauer F, Garg N, et al. Validity of the minimal assessment of cognitive function in multiple sclerosis (MACFIMS). *J Int Neuropsychol Soc*. (2006) 12:549–58. doi: 10.1017/S1355617706060723
8. Sanfilipo MP, Benedict RHB, Weinstock-Guttman B, Bakshi R. Gray and white matter brain atrophy and neuropsychological impairment in multiple sclerosis. *Neurology*. (2006) 66:685–92. doi: 10.1212/01.wnl.0000201238.93586.d9
9. Ayache SS, Palm U, Chalah MA, Nguyen R, Farhat WH, Créange A, et al. Orienting network dysfunction in progressive multiple sclerosis. *J Neurol Sci*. (2015) 351:206–7. doi: 10.1016/j.jns.2015.02.044
10. Chalah MA, Ayache SS. Deficits in Social Cognition: An Unveiled Signature of Multiple Sclerosis. *J Int Neuropsychol Soc*. (2017) 23:266–86. doi: 10.1017/S1355617716001156
11. Sumowski JF, Benedict R, Enzinger C, Filippi M, Geurts JJ, Hamalainen P, et al. Cognition in multiple sclerosis: state of the field and priorities for the future. *Neurology*. (2018) 90:278–88. doi: 10.1212/WNL.0000000000004977
12. Rao SM, Grafman J, DiGiulio D, Mittenberg W, Bernardin L, Leo GJ, et al. Memory dysfunction in multiple sclerosis: its relation to working memory, semantic encoding, and implicit learning. *Neuropsychology*. (1993) 7:364–74. doi: 10.1037/0894-4105.7.3.364
13. Guimarães J, Sá MJ. Cognitive dysfunction in multiple sclerosis. *Front Neurol*. (2012) 3:74. doi: 10.3389/fneur.2012.00074
14. Nebel K, Wiese H, Seyfarth J, Gizewski ER, Stude P, Diener HC, et al. Activity of attention related structures in multiple sclerosis patients. *Brain Res*. (2007) 1151:150–60. doi: 10.1016/j.brainres.2007.03.007
15. Archibald CJ, Fisk JD. Information processing efficiency in patients with multiple sclerosis. *J Clin Exp Neuropsychol*. (2000) 22:686–701. doi: 10.1076/1380-3395(200010)22:5;1-9;FT686
16. Green MF, Penn DL, Bentall R, Carpenter WT, Gaebel W, Gur RC, et al. Social cognition in schizophrenia: an NIMH workshop on definitions, assessment, and research opportunities. *Schizophr Bull*. (2008) 34:1211–20. doi: 10.1093/schbul/sbm145
17. Mike A, Strammer E, Aradi M, Orsi G, Perlaki G, Hajnal A, et al. Disconnection mechanism and regional cortical atrophy contribute to impaired processing of facial expressions and theory of mind in multiple sclerosis: a structural MRI study. *PLoS ONE*. (2013) 8:e82422. doi: 10.1371/journal.pone.0082422
18. Chalah MA, Kauv P, Lefaucheur JP, Hodel J, Créange A, Ayache SS. Theory of mind in multiple sclerosis: a neuropsychological and MRI study. *Neurosci Lett*. (2017) 658:108–13. doi: 10.1016/j.neulet.2017.08.055
19. Cotter J, Muhlert N. White matter changes and social cognitive function in MS: When all is no longer in the eyes. *Neurology*. (2017) 89:16–7. doi: 10.1212/WNL.0000000000004069
20. Cotter J, Firth J, Enzinger C, Kontopantelis E, Yung AR, Elliott R, et al. Social cognition in multiple sclerosis. *Neurology*. (2016) 87:1727–36. doi: 10.1212/WNL.0000000000003236
21. Yamout B, Issa Z, Herlopian A, El Bejjani M, Khalifa A, Ghadieh AS, et al. Predictors of quality of life among multiple sclerosis patients: a comprehensive analysis. *Eur J Neurol*. (2013) 20:756–64. doi: 10.1111/ene.12046
22. Kalb R, Beier M, Benedict RH, Charvet L, Costello K, Feinstein A, et al. Recommendations for cognitive screening and management in multiple sclerosis care. *Mult Scler*. (2018) 24:1665–80. doi: 10.1177/1352458518803785
23. Rocca MA, Amato MP, De Stefano N, Enzinger C, Geurts JJ, Penner IK, et al. Clinical and imaging assessment of cognitive dysfunction in multiple sclerosis. *Lancet Neurol*. (2015) 14:302–17. doi: 10.1016/S1474-4422(14)70250-9
24. Lefaucheur JP, Antal A, Ayache SS, Benninger DH, Brunelin J, Cogiamanian F, et al. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). *Clin Neurophysiol*. (2017) 128:56–92. doi: 10.1016/j.clinph.2016.10.087
25. Mattioli F, Bellomi F, Stampatori C, Capra R, Miniussi C. Neuroenhancement through cognitive training and anodal tDCS in multiple sclerosis. *Mult Scler*. (2016) 22:222–30. doi: 10.1177/1352458515587597
26. Charvet L, Shaw M, Dobbs B, Frontario A, Sherman K, Bikson M, et al. Remotely supervised transcranial direct current stimulation increases the benefit of at-home cognitive training in multiple sclerosis. *Neuromodulation*. (2018) 21:383–9. doi: 10.1111/ner.12583
27. Ayache SS, Lefaucheur JP, Chalah MA. Long term effects of prefrontal tDCS on multiple sclerosis fatigue: a case study. *Brain Stimul*. (2017) 10:1001–2. doi: 10.1016/j.brs.2017.05.004
28. Clayton AM, Howard J, Dobbs B, Shaw MT, Charvet LE. Remotely supervised transcranial direct current stimulation after ECT improves mood and cognition in a patient with multiple sclerosis: a case study. *J ECT*. (2018) 34:e15. doi: 10.1097/YCT.0000000000000474
29. Chalah MA, Lefaucheur JP, Ayache SS. Long-term effects of tDCS on fatigue, mood and cognition in multiple sclerosis. *Clin Neurophysiol*. (2017) 128:2179–80. doi: 10.1016/j.clinph.2017.08.004
30. Chalah MA, Grigorescu C, Padberg F, Kümpfel T, Palm U, Ayache SS. Bifrontal transcranial direct current stimulation modulates fatigue in multiple sclerosis: a randomized sham-controlled study. *J Neural Transm*. (2020) 127:953–61. doi: 10.1007/s00702-020-02166-2

31. Fonteneau C, Redoute J, Haesebaert F, Le Bars D, Costes N, Suaud-Chagny MF, et al. Frontal transcranial direct current stimulation induces dopamine release in the ventral striatum in human. *Cereb Cortex.* (2018) 28:2636–46. doi: 10.1093/cercor/bhy093
32. Fukai M, Bunai T, Hirosawa T, Kikuchi M, Ito S, Minabe Y, et al. Endogenous dopamine release under transcranial direct-current stimulation governs enhanced attention: a study with positron emission tomography. *Transl Psychiatr.* (2019) 9:115. doi: 10.1038/s41398-019-0443-4
33. Nissim NR, O'Shea A, Indahlastari A, Kraft JN, von Mering O, Aksu S, et al. Effects of transcranial direct current stimulation paired with cognitive training on functional connectivity of the working memory network in older adults. *Front Aging Neurosci.* (2019) 11:340. doi: 10.3389/fnagi.2019.00340
34. Hulst HE, Goldschmidt T, Nitsche MA, de Wit SJ, van den Heuvel OA, Barkhof F, et al. rTMS affects working memory performance, brain activation and functional connectivity in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatr.* (2017) 88:386–94. doi: 10.1136/jnnp-2016-314224
35. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* (2018) 17:162–73. doi: 10.1016/S1474-4422(17)30470-2
36. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. (1983) 33:1444–52. doi: 10.1212/WNL.33.11.1444
37. Parmenter BA, Shucard JL, Benedict RH, Shucard DW. Working memory deficits in multiple sclerosis: comparison between the n-back task and the paced auditory serial addition test. *J Int Neuropsychol Soc*. (2006) 12:677–87. doi: 10.1017/S1355617706060826
38. Sumowski JF, Wylie GR, Deluca J, Chiaravalloti N. Intellectual enrichment is linked to cerebral efficiency in multiple sclerosis: functional magnetic resonance imaging evidence for cognitive reserve. *Brain.* (2010) 133:362–74. doi: 10.1093/brain/awp307
39. Brunoni AR, Vanderhasselt MA. Working memory improvement with non-invasive brain stimulation of the dorsolateral prefrontal cortex: a systematic review and meta-analysis. *Brain Cogn.* (2014) 86:1–9. doi: 10.1016/j.bandc.2014.01.008
40. Mulquiney PG, Hoy KE, Daskalakis ZJ, Fitzgerald PB. Improving working memory: exploring the effect of transcranial random noise stimulation and transcranial direct current stimulation on the dorsolateral prefrontal cortex. *Clin Neurophysiol.* (2011) 122:2384–9. doi: 10.1016/j.clinph.2011.05.009
41. Oliveira JF, Zanão TA, Valiengo L, Lotufo PA, Benseñor IM, Fregni F, et al. Acute working memory improvement after tDCS in antidepressant-free patients with major depressive disorder. *Neurosci Lett.* (2013) 537:60–4. doi: 10.1016/j.neulet.2013.01.023
42. Miller KM, Price CC, Okun MS, Montijo H, Bowers D. Is the n-back task a valid neuropsychological measure for assessing working memory?. *Arch Clin Neuropsychol.* (2009) 24:711–7. doi: 10.1093/arclin/acp063
43. Smith A. *Symbol Digit Modalities Test (SDMT)*. Los Angeles, CA: Western Psychological Services (1982).
44. Van Schependom J, D'hooghe MB, Cleynhens K, D'hooge M, Haelewyck MC, De Keyser J, et al. The symbol digit modalities test as sentinel test for cognitive impairment in multiple sclerosis. *Eur J Neurol.* (2014) 21:1219–25. doi: 10.1111/ene.12463
45. Baron-Cohen S, Jolliffe T, Mortimore C, Robertson M. Another advanced test of theory of mind: evidence from very high functioning adults with autism or asperger syndrome. *J Child Psychol Psychiatr.* (1997) 38:813–22. doi: 10.1111/j.1469-7610.1997.tb01599.x
46. Baron-Cohen S, O'Riordan M, Stone V, Jones R, Plaisted K. Recognition of faux pas by normally developing children and children with Asperger syndrome or high-functioning autism. *J Autism Dev Disord.* (1999) 29:407–18. doi: 10.1023/A:1023035012436
47. Baron-Cohen S, Wheelwright S, Hill J, Raste Y, Plumb I. The "reading the mind in the eyes" test revised version: a study with normal adults, and adults with asperger syndrome or high-functioning autism. *J Child Psychol Psychiatr.* (2001) 42:241–51. doi: 10.1111/1469-7610.00715
48. Poletti M, Enrici I, Adenzato M. Cognitive and affective theory of mind in neurodegenerative diseases: neuropsychological, neuroanatomical and neurochemical levels. *Neurosci Biobehav Rev.* (2012) 36:2147–64. doi: 10.1016/j.neubiorev.2012.07.004
49. Mezger E, Rauchmann B, Brunoni AR, Bulubas L, Thielscher A, Werle J, et al. Effects of bifrontal transcranial direct current stimulation on brain glutamate levels and resting state connectivity: multimodal MRI data for the cathodal stimulation site. *Eur Arch Psychiatr Clin Neurosci.* (2020). doi: 10.1007/s00406-020-01177-0. [Epub ahead of print].
50. Gandiga PC, Hummel FC, Cohen LG. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clin Neurophysiol.* (2006) 117:845–50. doi: 10.1016/j.clinph.2005.12.003
51. Roberts DL, Penn DL. *Social Cognition in Schizophrenia: From Evidence to Treatment*. Oxford: Oxford University Press (2013). doi: 10.1093/med:psych/9780199777587.001.0001
52. Hallerbäck MU, Lugnegård T, Hjärthag F, Gillberg C. The reading the mind in the eyes test: test-retest reliability of a swedish version. *Cogn Neuropsychiatr.* (2009) 14:127–43. doi: 10.1080/13546800902901518
53. Pfaltz MC, McAleese S, Saladin A, Meyer AH, Stoecklin M, Opwis K, et al. The reading the mind in the eyes test: test-retest reliability and preliminary psychometric properties of the German version. *Int J of Adv in Psyc Res.* (2013) 2:e1–9. doi: 10.1080/13546805.2012.721728
54. Tomczak M, Tomczak E. The need to report effect size estimates revisited. an overview of some recommended measures of effect size. *Trends Sport Sci.* (2014) 1:19–25.
55. Hill AT, Fitzgerald PB, Hoy KE. Effects of anodal transcranial direct current stimulation on working memory: a systematic review and meta-analysis of findings from healthy and neuropsychiatric populations. *Brain Stimul.* (2016) 9:197–208. doi: 10.1016/j.brs.2015.10.006
56. Marshall L, Mölle M, Siebner HR, Born J. Bifrontal transcranial direct current stimulation slows reaction time in a working memory task. *BMC Neurosci.* (2005) 6:23. doi: 10.1186/1471-2202-6-23
57. Brunoni AR, Zanao TA, Ferrucci R, Priori A, Valiengo L, de Oliveira JF, et al. Bifrontal tDCS prevents implicit learning acquisition in antidepressant-free patients with major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatr.* (2013) 43:146–50. doi: 10.1016/j.pnpbp.2012.12.019
58. Keshvari F, Pouretemad HR, Ekhtiari H. The polarity-dependent effects of the bilateral brain stimulation on working memory. *Basic Clin Neurosci.* (2013) 4:224–31.
59. Funahashi S, Bruce CJ, Goldman-Rakic PS. Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. *J Neurophysiol.* (1989) 61:331–49. doi: 10.1152/jn.1989.61.2.331
60. Funahashi S, Bruce CJ, Goldman-Rakic PS. Visuospatial coding in primate prefrontal neurons revealed by oculomotor paradigms. *J Neurophysiol.* (1990) 63:814–31. doi: 10.1152/jn.1990.63.4.814
61. Funahashi S, Bruce CJ, Goldman-Rakic PS. Neuronal activity related to saccadic eye movements in the monkey's dorsolateral prefrontal cortex. *J Neurophysiol.* (1991) 65:1464–83. doi: 10.1152/jn.1991.65.6.1464
62. Curtis CE, D'Esposito M. Persistent activity in the prefrontal cortex during working memory. *Trends Cogn Sci.* (2003) 7:415–23. doi: 10.1016/S1364-6613(03)00197-9
63. Bauer RH, Fuster JM. Delayed-matching and delayed-response deficit from cooling dorsolateral prefrontal cortex in monkeys. *J Comp Physiol Psychol.* (1976) 90:293–302. doi: 10.1037/h0087996
64. Goldman PS, Rosvold HE. Localization of function within the dorsolateral prefrontal cortex of the rhesus monkey. *Exp Neurol.* (1970) 27:291–304. doi: 10.1016/0014-4886(70)90222-0
65. Paulesu E, Frith CD, Bench CJ, Bottini G, Grasby PG, Frackowiak RSJ. Functional anatomy of working memory: the visuospatial sketchpad. *J Cereb Blood Flow Metab.* (1993) 1:552–7.
66. Salmon E, Van der Linden M, Collette F, Delfiore G, Maquet P, Degueldre C, et al. Regional brain activity during working memory tasks. *Brain.* (1996) 119:1617–25. doi: 10.1093/brain/119.5.1617
67. Zimmer H. Visual and spatial working memory: From boxes to networks. *Neurosci Biobehav Rev*. (2008) 32:1373–95. doi: 10.1016/j.neubiorev.2008.05.016
68. Wu YJ, Tseng P, Chang CF, Pai MC, Hsu KS, Lin CC, et al. Modulating the interference effect on spatial working memory by applying transcranial direct current stimulation over the right dorsolateral prefrontal cortex. *Brain Cognition.* (2014) 91:87–94. doi: 10.1016/j.bandc.2014.09.002

69. Giglia G, Brighina F, Rizzo S. Anodal transcranial direct current stimulation of the right dorsolateral prefrontal cortex enhances memory-guided responses in a visuospatial working memory task. *Funct Neurol.* (2014) 29:189–93.
70. Bogdanov M, Schwabe L. Transcranial stimulation of the dorsolateral prefrontal cortex prevents stress-induced working memory deficits. *J Neurosci.* (2016) 36:1429–37. doi: 10.1523/JNEUROSCI.3687-15.2016
71. Ayache SS, Palm U, Chalah MA, Al-Ani T, Brignol A, Abdellaoui M, et al. Prefrontal tDCS decreases pain in patients with multiple sclerosis. *Front Neurosci.* (2016) 10:147. doi: 10.3389/fnins.2016.00147
72. Chalah MA, Riachi N, Ahdab R, Mhalla A, Abdellaoui M, Créange A, et al. Effects of left DLPFC versus right PPC tDCS on multiple sclerosis fatigue. *J Neurol Sci.* (2017) 372:131–7. doi: 10.1016/j.jns.2016.11.015
73. Forbes CE, Grafman J. The role of the human prefrontal cortex in social cognition and moral judgment. *Annu Rev Neurosci.* (2010) 33:299–324. doi: 10.1146/annurev-neuro-060909-153230
74. Li MS, Du XD, Chu HC, Liao YY, Pan W, Li Z, et al. Delayed effect of bifrontal transcranial direct current stimulation in patients with treatment-resistant depression: a pilot study. *BMC Psychiatr.* (2019) 19:180. doi: 10.1186/s12888-019-2119-2
75. Nitsche MA, Koschack J, Pohlers H, Hullemann S, Paulus W, Happe S. Effects of frontal transcranial direct current stimulation on emotional state and processing in healthy humans. *Front Psychiatr.* (2012) 3:58. doi: 10.3389/fpsyt.2012.00058
76. Cotelli M, Adenzato M, Cantoni V, Manenti R, Alberici A, Enrici I, et al. Enhancing theory of mind in behavioural variant frontotemporal dementia with transcranial direct current stimulation. *Cogn Affect Behav Neurosci.* (2018) 18:1065–75. doi: 10.3758/s13415-018-0622-4
77. Adenzato M, Manenti R, Enrici I, Gobbi E, Brambilla M, Alberici A, et al. Transcranial direct current stimulation enhances theory of mind in Parkinson's disease patients with mild cognitive impairment: a randomized, double-blind, sham-controlled study. *Transl Neurodegener.* (2019) 8:1. doi: 10.1186/s40035-018-0141-9
78. Adams RB, Ambady N, Nakayama K, Shimojo S. *The Science of Social Vision*. New York, NY: Oxford University Press (2011). doi: 10.1093/acprof:oso/9780195333176.001.0001
79. Sellaro R, Nitsche MA, Colzato LS. The stimulated social brain: effects of transcranial direct current stimulation on social cognition. *Ann N Y Acad Sci.* (2016) 1369:218–39. doi: 10.1111/nyas.13098
80. Conson M, Errico D, Mazzarella E, Giordano M, Grossi D, Trojano L. Transcranial electrical stimulation over dorsolateral prefrontal cortex modulates processing of social cognitive and affective information. *PLoS ONE.* (2015) 10:e0126448. doi: 10.1371/journal.pone.0126448
81. Rêgo GG, Lapenta OM, Marques LM, Costa TL, Leite J, Carvalho S, et al. Hemispheric dorsolateral prefrontal cortex lateralization in the regulation of empathy for pain. *Neurosci Lett.* (2015) 594:12–6. doi: 10.1016/j.neulet.2015.03.042
82. Santiesteban I, Banissy MJ, Catmur C, Bird G. Enhancing social ability by stimulating right temporoparietal junction. *Curr Biol.* (2012) 22:2274–7. doi: 10.1016/j.cub.2012.10.018
83. Mai X, Zhang W, Hu X, Zhen Z, Xu Z, Zhang J, et al. Using tDCS to explore the role of the right Temporo-parietal junction in theory of mind and cognitive empathy. *Front Psychol.* (2016) 7:380. doi: 10.3389/fpsyg.2016.00380
84. Vogeley K. Two social brains: neural mechanisms of intersubjectivity. *Philos Trans R Soc Lond B Biol Sci.* (2017) 372:20160245. doi: 10.1098/rstb.2016.0245
85. Winker C, Rehbein MA, Sabatinelli D, Dohn M, Maitzen J, Wolters CH, et al. Noninvasive stimulation of the ventromedial prefrontal cortex modulates emotional face processing. *Neuroimage.* (2018) 175:388–401. doi: 10.1016/j.neuroimage.2018.03.067
86. Beglinger LJ, Gaydos B, Tangphao-Daniels O, Duff K, Kareken DA, Crawford J, et al. Practice effects and the use of alternate forms in serial neuropsychological testing. *Arch Clin Neuropsychol.* (2005) 20:517–29. doi: 10.1016/j.acn.2004.12.003
87. Goldberg TE, Harvey PD, Wesnes KA, Snyder PJ, Schneider LS. Practice effects due to serial cognitive assessment: Implications for preclinical Alzheimer's disease randomized controlled trials. *Alzheimers Dement.* (2015) 1:103–11. doi: 10.1016/j.dadm.2014.11.003
88. Benedict RH, Smerbeck A, Parikh R, Rodgers J, Cadavid D, Erlanger D. Reliability and equivalence of alternate forms for the symbol digit modalities test: implications for multiple sclerosis clinical trials. *Mult Scler.* (2012) 18:1320–5. doi: 10.1177/1352458511435717
89. Benedict RH, DeLuca J, Phillips G, LaRocca N, Hudson LD, Rudick R. Multiple sclerosis outcome assessments consortium. Validity of the symbol digit modalities test as a cognition performance outcome measure for multiple sclerosis. *Mult Scler.* (2017) 23:721–33. doi: 10.1177/1352458517690821
90. Benedict RH. Effects of using same- versus alternate-form memory tests during short-interval repeated assessments in multiple sclerosis. *J Int Neuropsychol Soc.* (2005) 11:727–36. doi: 10.1017/S1355617705050782
91. Scharfen J, Jansen K, Holling H. Retest effects in working memory capacity tests: a meta-analysis. *Psychon Bull Rev.* (2018) 25:2175–99. doi: 10.3758/s13423-018-1461-6
92. Eddy CM. What do you have in mind? Measures to assess mental state reasoning in neuropsychiatric populations. *Front Psychiatr.* (2019) 10:425. doi: 10.3389/fpsyt.2019.00425
93. Cader S, Cifelli A, Abu-Omar Y, Palace J, Matthews PM. Reduced brain functional reserve and altered functional connectivity in patients with multiple sclerosis. *Brain.* (2006) 129:527–37. doi: 10.1093/brain/awh670
94. Vacchi L, Rocca MA, Meani A, Rodegher M, Martinelli V, Comi G, et al. Working memory network dysfunction in relapse-onset multiple sclerosis phenotypes: A clinical-imaging evaluation. *Mult Scler.* (2017) 23:577–87. doi: 10.1177/1352458516656809
95. Matías-Guiu JA, Cortés-Martínez A, Montero P, Pytel V, Moreno-Ramos T, Jorquera M, et al. Identification of cortical and subcortical correlates of cognitive performance in multiple sclerosis using voxel-based morphometry. *Front Neurol.* (2018) 9:920. doi: 10.3389/fneur.2018.00920
96. Reicker LI, Tombaugh TN, Walker L, Freedman MS. Reaction time: an alternative method for assessing the effects of multiple sclerosis on information processing speed. *Arch Clin Neuropsychol.* (2007) 22:655–64. doi: 10.1016/j.acn.2007.04.008
97. Palm U, Kumpf U, Behler N, Wulf L, Kirsch B, Wörsching J, et al. Home use, remotely supervised, and remotely controlled transcranial direct current stimulation: a systematic review of the available evidence. *Neuromodulation.* (2018) 21:323–33. doi: 10.1111/ner.12686

Conflict of Interest: FP is a member of the European Scientific Advisory Board of Brainsway Inc., Jerusalem, Israel, and has received speaker's honoraria from Mag & More GmbH and the neuroCare Group. His lab has received support with equipment from neuroConn GmbH, Ilmenau, Germany, and Mag & More GmbH and Brainsway Inc., Jerusalem, Israel. SSA declares having received travel grants or compensation from Genzyme, Biogen, Novartis and Roche. UP has a private practice with NeuroCare Group, Munich, Germany. MAC declares having received compensation from Janssen Global Services LLC. TK has received travel expenses and speaker honoraria from Bayer Vital, Teva Pharma, Merck, Novartis Pharma, Sanofi-Aventis/Genzyme, CLS Behring, Roche Pharma and Biogen as well as grant support/donation from Bayer-Schering AG, Novartis and Chugai Pharma.

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

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership