Heliyon 10 (2024) e35834

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Lowered ratio of corticospinal excitation to inhibition predicts greater disability, poorer motor and cognitive function in multiple sclerosis

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ARTICLE INFO

Keywords:
Multiple sclerosis
Excitatory-inhibitory (im)balance
Transcranial magnetic stimulation
Cortical silent period
Motor evoked potential
Brain asymmetry

ABSTRACT

Objective: Investigate excitatory-inhibitory (E/I) (im)balance using transcranial magnetic stimulation (TMS) in individuals with Multiple Sclerosis (MS) and determine its validity as a neurophysiological biomarker of disability.

Methods: Participants with MS (n = 83) underwent TMS, cognitive, and motor function assessments. TMS-induced motor evoked potential amplitudes (excitability) and cortical silent periods (inhibition) were assessed bilaterally through recruitment curves. The E/I ratio was calculated as the ratio of excitation to inhibition.

Results: Participants with greater disability (Expanded Disability Status Scale, EDSS$\geq$3) exhibited lower excitability and increased inhibition compared to those with lower disability (EDSS<3). This resulted in lower E/I ratios in the higher disability group. Individuals with higher disability presented with asymmetrical E/I ratios between brain hemispheres, a pattern not present in the group with lower disability. In regression analyses controlling for demographics, lowered TMS-probed E/I ratio predicted variance in disability ($R^2 = 0.37$, $p < 0.001$), upper extremity function ($R^2 = 0.35$, $p < 0.001$), walking speed ($R^2 = 0.22$, $p = 0.005$), and cognitive performance ($R^2 = 0.25$, $p = 0.007$). Receiver Operating Characteristic curve analysis confirmed 'excellent' discriminative ability of the E/I ratio in distinguishing high and low disability. Finally, excitation superiorly correlated with the E/I ratio than overall inhibition in both hemispheres ($p \leq 0.01$).

Conclusion: The E/I ratio is a potential neurophysiological biomarker of disability level in MS, especially when assessed in the hemisphere corresponding to the weaker body side. Interventions aimed at increasing cortical excitation or reducing inhibition may restore E/I balance potentially stalling progression or improving function in MS.

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https://doi.org/10.1016/j.heliyon.2024.e35834
Received 19 January 2024; Received in revised form 2 August 2024; Accepted 5 August 2024
Available online 6 August 2024

1. Introduction

Neuronal excitatory-inhibitory (E/I) balance is a key factor in regulating brain connectivity [1–5]. E/I balance describes the ratio between neuronal activity mediated by the major excitatory and inhibitory neurotransmitters glutamate and gamma-aminobutyric acid (GABA), respectively [1–5]. An elevated E/I ratio represents an imbalance towards enhanced cortical glutamatergic-mediated excitation and/or lowered GABA-mediated inhibition [1–5]. In pre-clinical models, abnormal E/I balance is linked to Autism Spectrum Disorders, Epilepsy, Schizophrenia, Rett syndrome, Alzheimer's, and Multiple Sclerosis (MS) [3–7]. Knocking out genes *in vivo* that express neuronal receptors associated with E/I imbalance can revert disease-like behaviors [4]. While it is not feasible to knockout genes in the human brain, these findings highlight the possibility of reversing the E/I imbalance in neurological disorders [8]. Studying E/I (im)balance in humans is of particular importance and could lead to the development of innovative treatments for neurological disorders.

MS is a neurodegenerative disease [9,10] resulting in accumulation of sensorimotor and cognitive symptoms to varying degrees [11]. Current pharmacological treatments do not cure MS; instead, they reduce the overactive immune response to mitigate MS attacks (i.e., relapses) and potentially slow disease progression [9]. Yet, 80 % of people initially diagnosed with relapsing-remitting MS will progress to advanced disease stages characterized by greater disability [9,12,13]. In models of MS, emergence of E/I imbalance in the somatosensory cortex may play a role in disease progression [7]. Although research investigating E/I in people with MS is lacking, several transcranial magnetic stimulation (TMS) studies report abnormalities in either excitatory and/or inhibitory circuitry in the primary motor areas among people with MS [14–25], supporting the plausible existence of an E/I imbalance in the MS brain. For example, increased levels of circulating pro-inflammatory cytokines in people with MS associate with both heightened disease activity and TMS metrics of enhanced glutamatergic receptor activity [21,24]. Conversely, the onset of progressive phases of MS and greater disability [26,27] accompanies diminished TMS-assessed excitability, and increased inhibition [15,20]. While previous research has focused on excitation and/or inhibition separately, using TMS to specifically investigate E/I (im)balance has not been explored in clinical populations. TMS offers a potential avenue for identifying neurophysiological biomarkers in MS [15,28,29], which is crucial to assist with disease progression detection and guide development of effective treatments [30].

The goal of this work was to use TMS to probe E/I (im)balance in people with MS. This approach is founded in work by Orth and Rothwell (2004), whereby the relationship between motor evoked potential (MEP) amplitude and cortical silent period (CSP) was first described [31]. MEP amplitude and CSP are sensitive to TMS stimulus intensity, such that the higher the TMS intensity, the higher the MEP amplitude and the longer the CSP [31]. MEP amplitudes are used to measure glutamatergic-receptor activity, whereas CSP, an interruption of electromyography activity in a tonic contracting muscle following a TMS-induced MEP, is a measure of GABAergic-receptor activity [29,31,32]. Orth and Rothwell (2004) proposed that cortical inhibition, measured using CSP, should only be considered abnormally increased in the event of disruption of the MEP-CSP relationship. Therefore, we applied this conceptual approach as a method for studying TMS-probed E/I (im)balance and we specifically examined: 1) whether people with MS presenting with higher disability had different E/I (im)balances when compared to people with MS with lower disability and the E/I differences between hemispheres across these two groups, and 2) the validity of this TMS-probed E/I as a biomarker of disability and physical and cognitive performance in MS. Our previous TMS research underscore the predictive potential of various TMS biomarkers in MS, particularly when measured within the brain hemisphere most impacted by the disease [15,16,18,33–35]. These findings demonstrate an asymmetry in corticospinal excitability, notably during advanced disability stages. Conversely, individuals with MS at lower disability levels demonstrate a more symmetrical corticospinal excitability [15,18]. Based on this evidence, we hypothesized that 1) people with higher MS disability would present lower E/I ratios due to lower excitation and excessive inhibition and E/I imbalance would be more pronounced in the hemisphere corresponding to the weaker side of the body (e.g., most affected side) and 2) lower E/I ratios would predict higher disability and poorer physical and cognitive function. Finally, to provide insight into the circuitry (excitation/inhibition) most associated with the E/I, we explored the associations between excitation (MEP) and inhibition (CSP), separately, with the derived E/I variable and we compared their correlation coefficients. Ultimately, this work could provide the field with a new neurophysiological biomarker of disability/disease progression in MS and inform the development of novel therapies, particularly non-invasive brain stimulation, that can potentially modify E/I (im)balance by targeting either excitatory and/or inhibitory circuitries and become part of an arsenal of yet-to-be-developed treatments for MS progression.

2. Methods

2.1. Participants and procedures

Participants (≥18 years-old) with a confirmed MS diagnosis (revised McDonald Criteria [36]) were sequentially recruited from their appointment with an MS neurologist at an MS Clinic. Inclusion criteria were: 1) able to walk indoors with or without walking assistive devices (e.g., cane, walker, wheelchair), 2) ≥18 years old, 3) no recent MS attacks (i.e., ≥3 months relapse-free), 4) able to undergo TMS as per standard screening form [37], and 5) no history of epilepsy or other contraindications to undergo TMS (e.g., metal/implants around the head/neck region). After screening, participants provided written informed consent and then completed a testing battery. In brief, participants were first given a package of standardized questionnaires, then tested for cognitive and upper extremity function, followed by TMS, and finally walking tests and assessment of cardiorespiratory fitness (refer to the supplementary material for all forms and questionnaires utilized). Other demographic and clinical data were extracted from medical records, including age (years), biological sex (female, male), MS type [relapsing-remitting, secondary progressive, or primary progressive MS], disease duration (years), and type of prescribed disease-modifying therapy. This study was approved by the Human Research Ethics

Board at Memorial University of Newfoundland (#20161208) and was carried out in accordance with the Declaration of Helsinki. This study is conducted in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines [38].

2.2. Disease disability, cognitive, and motor function outcomes

Disability status scores [Expanded Disability Status Scale (EDSS, 0–10)] were measured by the MS neurologist and retrieved from medical chart reviews [39]. Cognitive function was assessed using the written version of the Symbol Digit Modalities Test (SDMT) [40,41]. Upper extremity motor function was assessed using the Nine Hole Peg Test (9HPT) [42,43]. Lower extremity function was assessed as self-selected walking speed on a 4-m-long instrumented walkway (Protokinetics, Havertown PA, USA). Participants were instructed to walk twice across the instrumented walkway and the computed averaged velocity (cm/s) of the two trials was recorded. The height-corrected walking speed (cm/s/height$_{cm}$) was used to account for the relationship between height and walking speed [44].

2.3. Transcranial magnetic stimulation

Description of the TMS protocol and post-processing is described elsewhere [15] and in supplemental material. MEP amplitudes and CSPs were assessed in a recruitment curve (REC) [15]. As previously suggested, collecting MEPs and CSPs in a REC experiment can provide a better understanding of the overall magnitude of cortical excitation and inhibition [31,32]. Grip and pinch maximal voluntary contractions were used to discriminate the weaker and stronger hands [15] and clinically determine the most and least affected hemisphere [45], respectively. Making this distinction when collecting TMS variables is crucial due to hemispheric differences in cortical excitability, distinct symptom associations, and neuroplastic capacity in MS [15,16,18,34,35]. As proposed by Orth and Rothwell [31], and similar to other neuroimaging and electrophysiology approaches (e.g., magnetic resonance spectroscopy, electroencephalogram) [46], the E/I (im)balance was computed as a ratio between excitation and inhibition [i.e., E/I ratio = MEP amplitudes, excitatory REC (eREC)/CSP times, inhibitory REC (iREC)].

2.4. Statistical analysis

Detailed information on data reporting, test statistics, assumptions, and data transformation can be found in the supplementary material.

2.5. Between-group differences on demographics, RECs, and E/I ratios

A General Linear Model, two-group multivariate analysis of variance (Hotelling's T^2) [47,48] with post hoc were performed to determine differences between groups (EDSS$\geq$3 vs EDSS$<$3; independent variable) on the dependent variables; demographics [age and age of MS onset (years-old), and disease duration (years)], eREC, iREC and the E/I ratios from both hemispheres. Three Hotelling's T^2 were performed with respect to the research questions and the level of significance was Bonferroni-adjusted (p-value = 0.05/n of dependent variables) accordingly [i.e., to $p < 0.017$ for demographics, to $p < 0.013$ for eREC and iREC, and to $p < 0.025$ for E/I ratios]. Differences between groups are reported as mean differences with 95 % confidence intervals (CI). Chi-squared tests of homogeneity investigated the difference between the proportion (%) of males and females (biological sex) and participants taking disease-modifying drugs (prescribed, not prescribed).

2.6. Within-group differences in hemispheric E/I ratios

The E/I ratios were compared between the two hemispheres using paired t-tests. This analysis was performed with all participants together, and in the higher (EDSS$\geq$3) and lower disability (EDSS$<$3) groups separately. The significance level was set at $p < 0.05$.

2.7. E/I ratio as a biomarker of disability in MS

Pearson's correlations were performed between the E/I ratios from both hemispheres and disability status (EDSS), cognitive (SDMT) and motor performance (9HPT and walking speed). Significant relationships ($p < 0.05$) were further investigated with hierarchical regression analyses controlling for age, disease-modifying drug use, and sex, as previous research has shown their impact on corticospinal excitability and MS progression [16,22,29]. Additionally, a Receiver Operating Characteristic (ROC) curve analysis was performed to investigate the ability of the E/I ratio to classify high and low disability (EDSS$\geq$3 and $<$ 3, respectively; dependent variable).

2.8. Correlation between excitation and inhibition with E/I ratio

To elucidate the contribution of excitation and inhibition, separately, to the excitatory-inhibitory ratio, Pearson's correlation coefficients were performed and compared between the overall excitation and inhibition (eREC and iREC, respectively) and E/I ratios.

Additional independent t-tests were conducted to explore sex differences in TMS (iREC, eREC, and E/I ratio), disability levels (EDSS), and physical (9HPT, walking speed) and cognitive function (SDMT) between the two biological sexes (female, male).

Furthermore, based on previous studies examining sex-specific associations between cognitive function and brain excitability assessed with TMS [16], Pearson's correlations were calculated between this new TMS biomarker, the E/I ratio, and cognitive function (SDMT), stratified by sex (male, female).

3. Results

3.1. Participants

We approached 223 people with MS, and 192 people consented to participate. TMS assessment was a later addition to this longitudinal study's protocol. Initially, 110 participants were assessed for TMS motor thresholds only. The recruitment curve experiments were subsequently added to this protocol and were assessed in 83 participants (refer to supplementary material for STROBE flow diagram).

Descriptive data are reported in Table 1. There were differences between disability groups on demographics ($F_{(3, 77)} = 3.25$, Wilk'sΛ = 0.88, $p = 0.026$, partial $\eta 2 = 0.11$) and performance scores ($F_{(3, 70)} = 14.20$, Wilk'sΛ = 0.62, $p < 0.001$, partial $\eta 2 = 0.38$). Participants in the high disability group (EDSS≥3) were older ($p = 0.022$), had a longer disease duration ($p = 0.005$), and performed worse on both cognitive (SDMT; $p = 0.002$) and motor assessments (walking speed and 9HPT, both $p < 0.001$) compared to those in the low disability group (EDSS>3.0).

All TMS values (mean ± SD) and comparisons are reported in Table 2.

3.2. Lower excitation, higher inhibition, and lower E/I ratio in high disability group

There was a difference between low (EDSS<3) and high (EDSS≥3) disability groups when comparing the overall excitation and inhibition (eREC AUC and iREC AUC; $F_{(4, 66)} = 6.66$, Wilk'sΛ = 0.712, $p < 0.001$) and the E/I ratios ($F_{(2, 68)} = 9.89$, Wilk'sΛ = 0.775, $p < 0.001$) across brain hemispheres. When compared to the low disability group, the high disability group presented with lower overall excitation (eREC AUC) and higher overall inhibition (iREC AUC) in both hemispheres ($p < 0.05$) (Table 2). However, adjusted statistical significance was reached only when comparing the hemisphere corresponding to the weaker hand (eREC AUC: $p < 0.001$, mean difference −0.29, 95 % CI = −0.45 to −0.14; iREC AUC: $p = 0.006$, mean difference +0.13 95%CI = +0.12 to +0.25). Lower overall excitation (eREC AUC) combined with higher overall inhibition (iREC AUC) explained the lower E/I ratios in both hemispheres in the high disability group (strong hand: $p = 0.007$, mean difference −0.792, 95%CI = −1.45 to −0.138; weak hand: $p < 0.001$, mean difference = −1.25, 95%CI = −1.89 to −0.60; Fig. 1A and B).

3.3. The E/I ratio was asymmetrical between hemispheres in the higher disability group

When all participants were analyzed together, the E/I ratio measured in the hemisphere corresponding to the stronger hand was higher compared to the hemisphere corresponding to the weaker hand ($t_{(70)} = 2.63$, $p = 0.010$; Fig. 1C). When disability groups were examined, this difference did not exist in the low disability group (EDSS<3; $t_{(53)} = 1.51$, $p = 0.138$; Fig. 1A), but remained significant in the high disability group (EDSS≥3; $t_{(16)} = 2.89$, $p = 0.011$; Fig. 1B). Fig. 2 summarizes the abovementioned findings and suggests a profile of lowered and brain asymmetrical E/I ratio in high disability group (EDSS≥3).

3.4. Lower E/I ratios predicted greater disability and poorer cognitive and motor performance

Lower E/I ratios from both hemispheres correlated with higher disability scores (EDSS; Fig. 3A) and with poorer motor and

Table 1
Participants demographics.

	All Participants (n = 83)	Low Disability (EDSS <3; n = 59)	High Disability (EDSS ≥3; n = 24)
Age (years-old)	48.99 ± 10.3	47.37 ± 10.0	52.96 ± 10.2*
EDSS (0-10) [median (range)]	2.5 (0–6.5)	1.0 (0–2.5)	6.0 (3.0–7.0)
MS Type (RRMS/SPMS/PPMS)	67/11/5	58/0/1	9/11/4
Age of MS onset (years-old)	34.04 ± 9.3	33.98 ± 8.0	34.17 ± 11.9
Disease Duration (years)	15.04 ± 8.1	13.44 ± 7.8	18.83 ± 7.8**
Walking Speed (cm/s; cm/s/height$_{cm}$)	101.02 ± 30.5; 0.60 ± 0.2	113.27 ± 20.8; 0.67 ± 0.1	70.90 ± 30.1; 0.42 ± 0.2***
9HPT (s)	23.10 ± 6.1	21.44 ± 4.1	29.25 ± 8.0***
SDMT	48.21 ± 11.0	50.45 ± 10.1	40.59 ± 10.8**
DMD (%, prescribed/not prescribed)	65.1 %/34.9 %	72.9 %/27.1 %	54.2 %/45.8 %*
Sex (%, males/females); Female-to-male ratio	27.3 %/72.7 %; 3:1	25 %/75 %; 3:1	32.4 %/67.6 %; 2:1

Note: 9HPT, nine-hole peg test; EDSS, expanded disability status scale; DMD, disease-modifying drugs; MS, multiple sclerosis; RRMS, relapsing remitting MS, SPMS, secondary progressive MS, PPMS, primary progressive MS; *, $p < 0.050$; **, $p < 0.010$; ***, $p < 0.001$ (adjusted p-values). All values are reported as mean ± standard deviation, with exception of DMD and Sex (%, proportion). In the total sample, fifty-four participants (65.1 %) were prescribed DMDs. Twenty-three participants were on dimethyl fumarate, 10 on glatiramer acetate, 9 on fingolimod, 4 on interferon-beta 1a, 4 on teriflunomide, 2 on cladribine, 1 on alemtuzumab, and 1 on natalizumab. There was a higher proportion of participants being prescribed DMDs in the lower disability (EDSS <3; 72.9 %) compared to the higher disability (EDSS ≥3; 54.2 %) group ($p = 0.018$).

Table 2
Transcranial magnetic stimulation (TMS) – overall excitation, inhibition, and E/I ratio.

TMS variable	All participants		Low Disability (EDSS <3)		High Disability (EDSS ≥3)	
	Strong Hand	Weak Hand	Strong Hand	Weak Hand	Strong Hand	Weak Hand
AMT (MSO%)	34 ± 8	38 ± 14	33 ± 7	34 ± 9	38 ± 9	48 ± 18
eREC AUC; log. Transformed†	61512.9 ± 33721.6; 4.72 ± 0.25	55249.2 ± 28495.4; 4.68 ± 0.25	64844.1 ± 32683.0; 4.76 ± 0.22	62638.8 ± 27611.1; 4.75 ± 0.20	52579.1 ± 35594.7; 4.62 ± 0.32£	34558.14 ± 19665.9; 4.47 ± 0.24***
iREC AUC; log. Transformed†	5289.0 ± 1890.8; 3.70 ± 0.16	5738.8 ± 2267.0; 3.73 ± 0.17	4940.28 ± 1728.8; 3.67 ± 0.15	5225.58 ± 1849.0; 3.69 ± 0.16	6208.3 ± 2028.57; 3.77 ± 0.15£	7150.18 ± 2727.7; 3.82 ± 0.19**
E/I Ratio††	3.46 ± 1.11	3.23 ± 1.12€	3.67 ± 1.02	3.54 ± 0.98	2.89 ± 1.14*	2.33 ± 1.01€**

Note: AMT, active motor threshold [used for normalization of recruitment curve (REC) experiment]; AUC, area under the curve; eREC, excitatory recruitment curve; iREC, inhibitory recruitment curve; EDSS, expanded disease disability status; E/I Ratio, excitatory-inhibitory ratio (eREC AUC/iREC AUC = E/I Ratio, and ††squared-transformed to reach distribution normality); †, log. Transformed data to reach distribution normality. Between-group differences at the Bonferroni-adjusted p-value: *, $p < 0.01$, **, $p < 0.005$, ***$p < 0.001$; £, Between-group difference at the non-adjusted Bonferroni p-value ($p < 0.05$). €, within-group differences between hemispheric E/I ratio ($p < 0.05$).

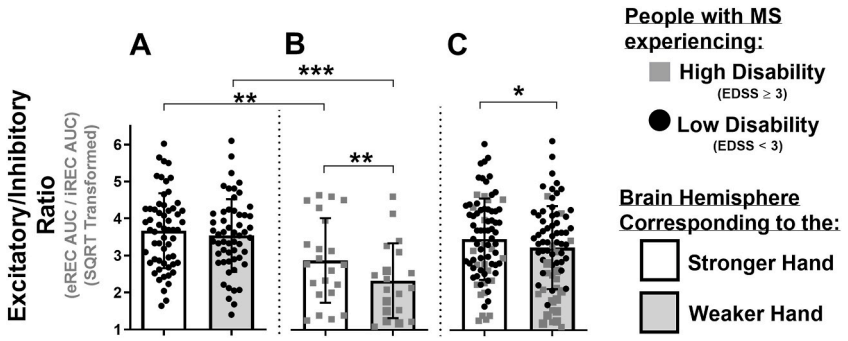

Fig. 1. Lower excitatory-inhibitory (E/I) ratio in high disability Multiple Sclerosis (MS). Graph reports the between- and within-group differences in E/I ratios. Between-group differences: **(A)** The low disability [expanded disability status scale (EDSS) < 3] had higher E/I ratios in both hemispheres when compared to the **(B)** high disability group (EDSS≥3). Within-group differences: **(C)** When all people with MS were analyzed together as one group, E/I ratios measured in the hemisphere corresponding to the stronger hand were higher compared to the hemisphere corresponding to the weaker hand. Analysis with the sample divided into low and high disability MS (EDSS<3 and EDSS≥3, respectively) revealed that **(A)** this difference between E/I ratios across hemispheres did not exist in the low disability group (EDSS<3), whereas **(B)** in the high disability group (EDSS≥3), the hemisphere corresponding to the weaker hand had a lower E/I ratio compared to the contralateral hemisphere. The E/I data (y-axis) was squared-root (SQRT) transformed in order to achieve normality of distribution. Significances at the Bonferroni-adjusted p-values *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$. Figure created on GraphPad Prism (V.8. San Diego, California USA). Bars represent the mean and the error lines represent the standard deviation.

cognitive performance scores (SDMT and 9HPT; Table 3). The relationships with the strongest correlation coefficients across the two hemispheres were further tested in hierarchical regression analysis. The E/I ratios contributed significantly to the model explaining variance in disease disability (EDSS; +24.4 %), upper extremity dexterity (9HPT; +17.3 %), walking speed (+12.6 %), and cognitive function (SDMT; +9.4 %). Results from correlation and regression analyses are presented in Table 3.

Results from the ROC curve revealed an optimal cut-off point of 2.61 (SQRT-transformed E/I ratio) and a respective sensitivity of 74 % and a specificity of 15 %, i.e., 74 % of cases will be correctly classified as high disability MS, with a 15 % chance of false positive. The area under the ROC curve had a value of 0.808 (95%CI 0.685–0.931; $p < 0.001$) indicating "excellent" classification ability (Fig. 3B).

In Fig. 4, depicting representative individual data, it is possible to visualize that the lower E/I ratios in the higher disability group resulted from a combination of both lower excitation (lower MEP amplitudes) and excessive inhibition (longer CSPs). Specifically, Fig. 4 shows raw MEP (used to compute eREC) and CSP signal traces (used to compute iREC) collected from two participants (same sex, similar ages, and different disability levels). In the participant with higher disability (EDSS = 6.0), MEP amplitudes increased slightly in response to increases in TMS stimulation intensity, whereas CSP continued to be prolonged (Fig. 4A). Conversely, the MS participant with lower disability (EDSS = 0) had shorter CSP times and much higher MEP amplitudes that continued to increase with increases in TMS stimulation intensity (Fig. 4B), which resulted in a much higher E/I ratio.

3.5. Relationships between excitation and inhibition with the E/I ratio

As expected, both excitation and inhibition (eREC AUC and iREC AUC, respectively) were strongly correlated with the E/I ratio in both hemispheres, whereby the lower the excitation and the higher the inhibition, the lower the E/I ratio (Fig. 5). In both hemispheres,

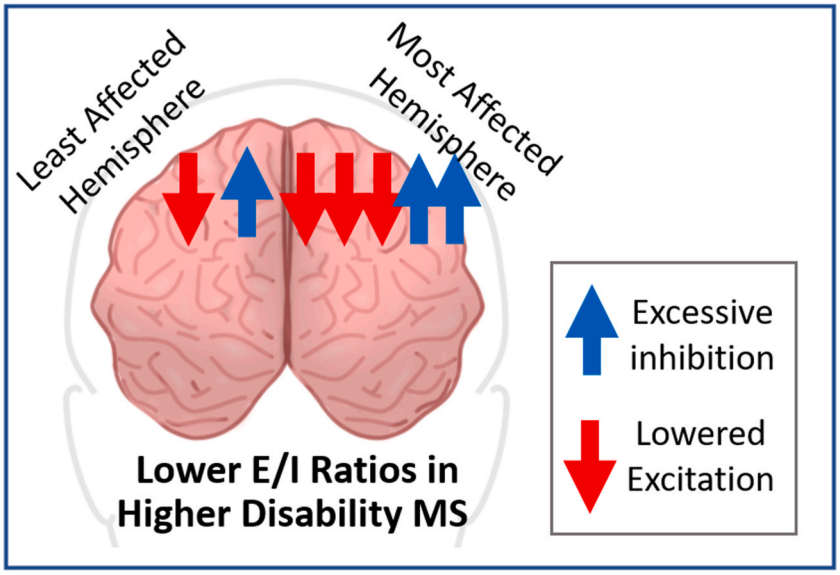

Fig. 2. Schematic of E/I ratio in the high disability group based on the within- and between-subject comparisons of overall excitation and inhibition. Higher disability group (EDSS≥3) presented with lower overall excitation (excitatory recruitment curve) and higher overall inhibition (inhibitory recruitment curve) in both hemispheres in comparison to the lower disability group (EDSS<3) at the $p < 0.05$ level of significance. Statistical significance at the Bonferroni-adjusted p-value was reached only when comparing the hemisphere corresponding to the weaker hand (eREC AUC: $p < 0.001$, iREC AUC: $p < 0.01$). The arrows in the suggested model represent the statistical differences between groups (EDSS<3 vs EDSS≥3) across hemispheres for eREC and iREC. One arrow in the right (representing the least affected) hemisphere represents a significance at the non-adjusted p-value. Two and three arrows in the left (representing the most affected hemisphere) represents significances at the at the adjusted p-value $p < 0.01$ and $p < 0.001$, respectively. Figure created on Autodesk® Sketchbook® free software.

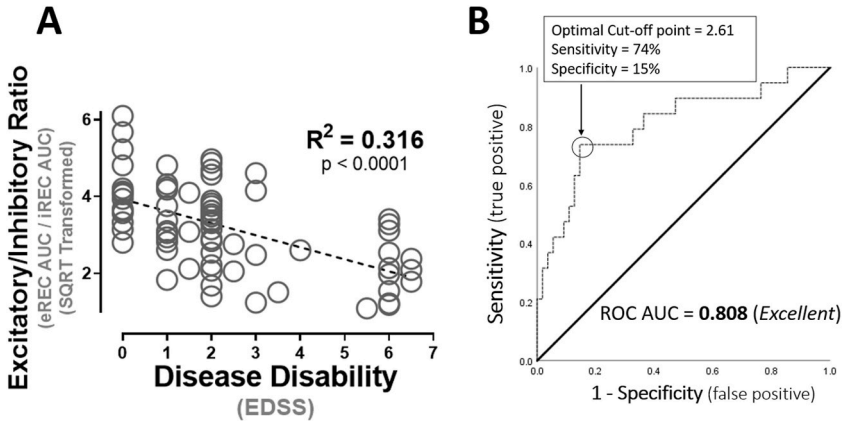

Fig. 3. The E/I ratio as a biomarker of disability progression in Multiple Sclerosis (MS). (A) Relationship between disease disability measured using the Expanded Disability Status Scale (EDSS) and excitatory-inhibitory (E/I) ratio measured in the hemisphere corresponding to the weaker hand. eREC, excitatory recruitment curve; iREC inhibitory recruitment curve; AUC, area under the curve. SQRT, squared-root; R-squared (R^2) reports the strength of the association. **(B)** Receiver operator characteristic area under the curve (ROC AUC) demonstrated 'excellent' ability of the excitatory-inhibitory (E/I) ratio on classifying high and low disability MS (expanded disease disability scale ≥3 and < 3, respectively). Solid circle indicates optimal cut-off point and respective sensitivity (true positive, y-axis) and specificity (false positive, x-axis) values. Figure A created on GraphPad Prism (V.8. San Diego, California USA), and Figure B on SPSS software.

overall excitation (eREC AUC) had a significantly stronger correlation with the E/I ratio (Fig. 5A and C) when compared to the correlation between inhibition (iREC AUC) and E/I ratio (Fig. 5B and D) (eREC AUC vs iREC AUC and E/I ratio: Strong hand: $|r| = 0.841$ vs $|r| = 0.556$; $Z = 3.85$, $p < 0.001$, Weak hand: $|r| = 0.853$ vs $|r| = 0.709$; $Z = 2.58$, $p = 0.01$.

Additional exploratory analyses investigating sex differences did not reveal any difference between males and females across any of the outcomes collected (TMS, or function; $t \leq 1.58$, $p \geq 0.126$).

In females, the E/I ratio measured in the hemisphere corresponding to the weaker hand was associated with SDMT ($r = 0.366$, $p = 0.008$) but not the E/I ratio measured in the hemisphere corresponding to the stronger hand ($r = 0.212$, $p = 0.121$). In males, the E/I

Table 3
Excitatory-inhibitory (E/I) ratio predicts disability, cognitive, and motor performance in MS.

Table 3. Excitatory-inhibitory (E/I) ratio predicts disability, cognitive, and motor performance in MS.					
	E/I ratio measured in the hemisphere corresponding to the:	**EDSS**	**SDMT**	**9HPT**	**Walking Speed**
1) Pearson's correlation coefficients	Stronger hand	r = -0.395, p < 0.001	r = 0.268, p = 0.022	r = -0.360, p = 0.002	r = -0.379, p < 0.001
	Weaker hand	r = -0.563, p < 0.001	r = 0.354, p = 0.003	r = -0.443, p < 0.001	r = -0.337, p = 0.003
2) Hierarchical Regression Analyses	**Outcome (predicted) variable:**	**Block 1: Controlling variables**	**Contribution of controlling variables (R^2, F-change and sig.)**	**Block 2: Addition of E/I ratio (ΔR^2, F-change and sig.)**	**= Final Model (R^2, F-value and sig. of final model)**
	EDSS	Age, MS onset, DD, DMD, and Sex.	12.5% ($F_{(5, 66)}$ = 1.98, p = 0.108)	**+24.4% ($F_{(1, 65)}$ = 25.21, p < 0.001)**	**37.0% ($F_{(6, 65)}$ = 6.35, p < 0.001)**
	SDMT		**15.8% ($F_{(5, 63)}$ = 2.37, p = 0.049**	**+9.4% ($F_{(1, 62)}$ = 2.37, p = 0.007**	**25.2% ($F_{(6, 62)}$ = 3.48, p = 0.005)**
	9HPT		**18.0% ($F_{(5, 63)}$ = 2.76, p = 0.026**	**+17.3 ($F_{(1, 62)}$ = 16.57, p < 0.001**	**35.3% ($F_{(6,62)}$ = 5.63, p < 0.001**
	Walking speed		9.6% ($F_{(5, 73)}$ = 1.55, p = 0.186)	**+12.6% (F, $_{(1, 72)}$ = 11.64, p = 0.001)**	**22.2% ($F_{(6, 72)}$ = 3.42, p = 0.005)**
Key Results: **Block 1:** Clinical demographics (controlling variables) added in the first block significantly predicted variance in SDMT and 9HPT, but not EDSS and Walking speed. **Block 2:** Addition of the E/I ratio in the second block contributed significantly to all models and rendered all final models statistically significant in explaining variance of the outcome variables (EDSS, SDMT, 9HPT, and Walking speed).					
Note: 9HPT, nine-hole peg test (average score from both hands); DD, disease duration (years), DMD, disease-modifying drugs (prescribed, not prescribed); EDSS, expanded disability status scale; SDMT, symbol digit modalities test; Sex, biological sex (female, male). Gray-shaded boxes (Pearson's, step 1) identify the correlations that were tested in hierarchical analysis (step 2); Significant models are highlighted in bold.					

Note: 9HPT, nine-hole peg test (average score from both hands); DD, disease duration (years), DMD, disease-modifying drugs (prescribed, not prescribed); EDSS, expanded disability status scale; SDMT, symbol digit modalities test; Sex, biological sex (female, male). Gray-shaded boxes (Pearson's, step 1) identify the correlations that were tested in hierarchical analysis (step 2); Significant models are highlighted in bold.

ratio measured in the hemisphere corresponding to the stronger hand was associated with SDMT ($r = 0.458$, $p = 0.042$), but not the E/I ratio measured in the hemisphere corresponding to the weaker hand ($r = 0.457$, $p = 0.056$).

4. Discussion

E/I balance regulates brain connectivity and is vital for optimal brain function [1–5]. The objective of this work was to probe E/I (im)balance using TMS in a cohort of people with MS with mild to severe disability. We report three main findings. First, TMS-probed E/I ratios were lower in people with MS who had higher disability (EDSS≥3) when compared to those with lower disability (EDSS<3). Lower E/I ratio values were explained by the lower overall excitation and higher overall inhibition in the high disability group, effects that were more pronounced in the hemisphere corresponding to the weaker side of the body, likely the side most affected [45]. Second, the E/I ratio explained variance in disability status (EDSS), upper extremity dexterity (9HPT), walking speed, and cognitive performance (SDMT), after controlling for demographics and clinical characteristics. In addition, the E/I ratio demonstrated an 'excellent' ability to differentiate between high and low disability levels in MS, as indicated by the ROC analysis. These results suggest the potential validity of the E/I ratio as a neurophysiological biomarker of disability in MS. Our findings confirm that E/I can be probed by analysis of the muscle contracting TMS-induced MEP signal and dividing its MEP amplitudes by their respective CSP times, as previously suggested by Orth and Rothwell [31]. Finally, in both hemispheres, overall excitation was more strongly correlated with the E/I ratio compared to overall inhibition, suggesting that excitation might play a more prominent role in the E/I imbalance in MS. Overall, these data suggest that increasing cortical excitation and/or reducing inhibition through targeted interventions might have the potential to reduce disability/stall progression by restoring E/I balance.

4.1. The E/I (im)balance as a biomarker of MS disability

In our study, the TMS-probed E/I ratios were lower in both hemispheres in the high disability (EDSS≥3) compared to the low

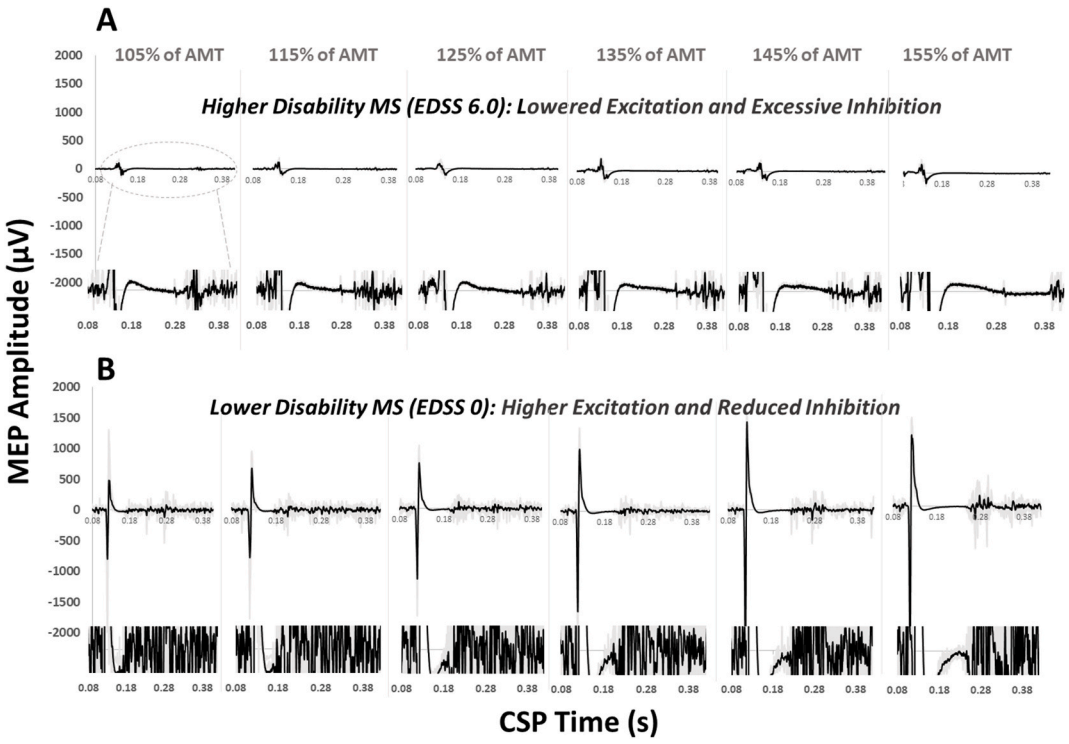

Fig. 4. Averaged raw signal from Transcranial Magnetic Stimulation (TMS) recruitment curves (REC) measured in the hemisphere corresponding to the weaker hand in two participants with Multiple Sclerosis (MS). (A) 46-year-old female with secondary progressive MS and a higher degree of disability [i.e., 6.0 on the Expanded Disability Status Scale (EDSS)]. In this participant, motor evoked potential (MEP) amplitudes (y-axis) increase slightly in response to increases in TMS stimulation intensity (*lowered excitation*), whereas CSP is prolonged (*excessive inhibition*). **(B)** 41-year-old female participant with relapsing-remitting MS and lower disability (EDSS 0). In this participant, higher MEP amplitudes increase accordingly with the increases in TMS stimulation intensity (*higher excitation*) with less prolongation of CSP (*reduced inhibtion*). The squared-root-transformed E/I ratio for participant A was 5.23 and for participant B was 1.18. Dotted circle/lines indicate that, in all signals, the y-axis (MEP amplitude) was reduced from ±2000 μV to ±20 μV for magnification of signal and better visualization of the CSP phenomena. The time, however, remained unchanged (y-axis, CSP time in seconds). RECs were collected with participants performing a slight contraction (pinching in a dynamometer) at 10 % of their maximal voluntary pinch contraction. TMS was delivered at six intensities 105 %, 115 %, 125 %, 135 %, 145 % and 155 % of the active motor threshold (AMT). Participants A and B had an AMT of 40 % and 58 % of the maximal stimulator output (MSO%), respectively. Figure created on Microsoft Excel (Redmond, WA, USA).

disability group (EDSS<3). Lower overall excitation (reduced MEPs) and higher overall inhibition (longer CSPs), explained the lower E/I ratios in participants with high disability (Fig. 4). Although there is a paucity of research investigating E/I (im)balance in MS, our results concur with previous TMS studies [17,49–52]. For example, reduced MEP amplitude and prolonged CSP was detected in people with MS when compared to healthy controls, and in people with MS with higher disability compared to those with lower disability [19,20,50,52–56]. Others confirmed the associations between reduced MEPs, prolonged CSP and increased MS-related disability [19,20,53,57,58] supporting the premise that excitatory and inhibitory circuitry are both affected by MS. Notably, previous studies have primarily focused on investigating either excitation or inhibition independently. To our knowledge, this is the first investigation integrating these two interconnected circuitries (excitation and inhibition) into a unified outcome measure, as TMS-probed E/I ratio, and confirmed its validity in MS.

4.2. The role of E/I (im)balance in motor and cognitive function

Fine motor control requires a balance between excitation and inhibition. Stinear, et, al. reviewed the complex interactions between the primary motor cortex and other cortical and subcortical brain regions subserving optimal voluntary motor movement and established the value of TMS to probe underlying intracortical excitation and inhibition [59]. They concluded that inhibitory dysfunction within the cortical–striatal–thalamic–cortical pathway is responsible for impaired motor planning and movement execution in some neurological conditions (e.g., focal dystonia, attention deficit hyperactivity disorder, and Tourette's syndrome) [59]. In other words, if a provided motor action requires greater corticospinal excitatory output, high inhibitory input to M1 should take place. In this context, excessive inhibition and/or too little excitation would interfere with voluntary movement. TMS findings reporting prolonged CSP and lower MEP amplitudes related to palsy-like symptoms in other conditions such as stroke, Huntington's, and Sydenham's chorea, suggest that this is the case [60–63]. Our findings of lowered E/I ratios predicting poorer motor function in

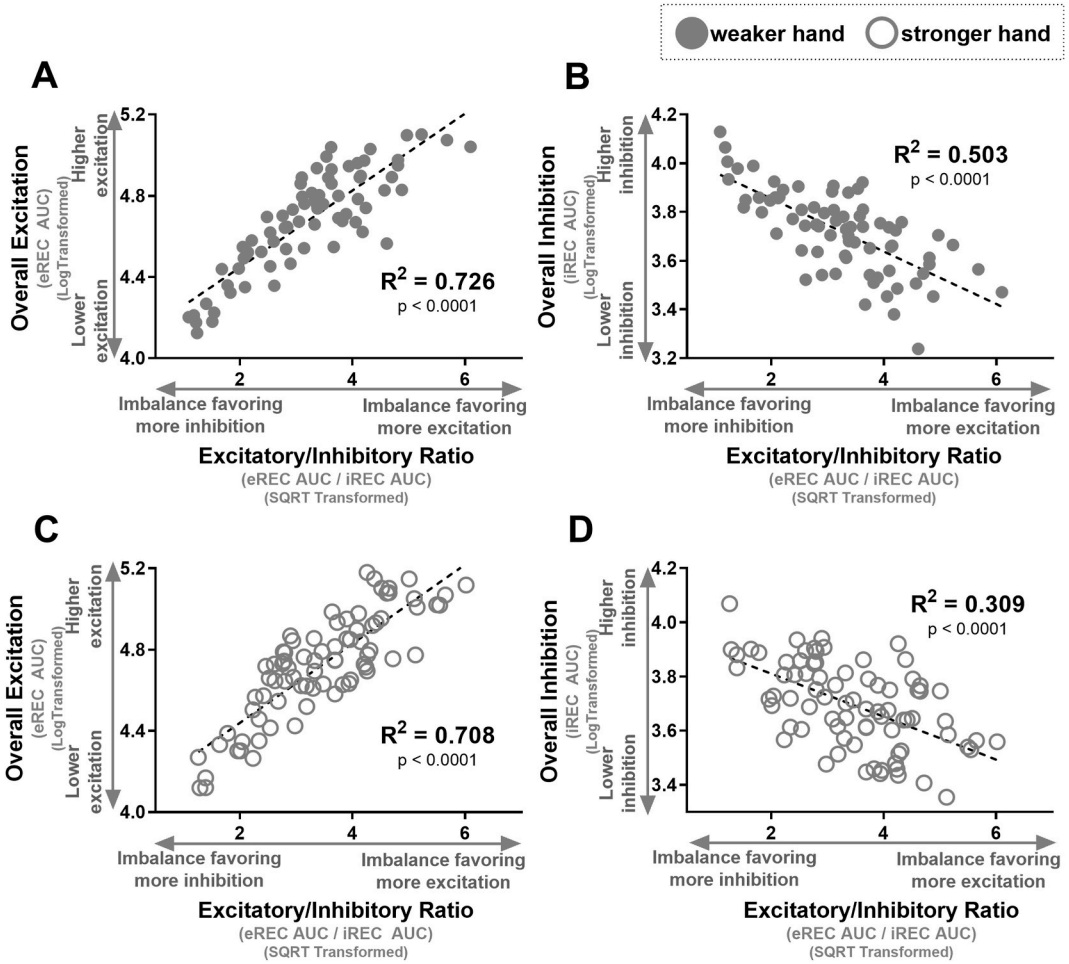

Fig. 5. Correlations between overall excitation and inhibition with excitatory/inhibitory (E/I) ratio. Within the hemisphere corresponding to the weaker hand, **(A)** overall excitation (excitatory recruitment curve, eREC) and **(B)** overall inhibition (inhibitory recruitment curve, iREC) were both correlated with the E/I ratio. There was a statistically significant difference whereby the correlation between overall excitation and E/I ratio was stronger than the correlation between overall inhibition and E/I ratio. Similarly, within the hemisphere corresponding to the stronger hand, **(C)** overall excitation and **(D)** overall inhibition were both correlated with the E/I ratio, and there was also a statistically significant difference whereby the correlation between overall excitation and E/I ratio was stronger than the correlation between overall inhibition and E/I ratio. Data were logarithmic (Log) or squared-root (SQRT) transformed in order to achieve normality of distribution and perform the parametric test (Pearson's correlation coefficients, *r*). The r-squared (R^2) reports the strength of associations. Figure created on GraphPad Prism Prism (V.8. San Diego, California USA).

MS align with previous work and strengthen the proposed role of E/I balance during voluntary movement proposed by Stinear et al. [59].

Predicting cognitive decline, a prevalent and disabling MS symptom, is a challenge [64]. In our study, lower E/I ratios predicted poorer performance on the SDMT, suggesting that E/I ratio has the potential to serve as an additional biomarker complementing techniques such as MRI [65]. A recent study reported that global cortical E/I imbalance towards increased inhibition, investigated with resting-state magnetoencephalography, was associated with poorer SDMT performance in people with MS [66]. In the paucity of clinical studies investigating E/I and cognitive function, experimental models of E/I dysfunction, pharmacological agents, and optogenetics have helped elucidate the link between E/I (im)balance and cognition [67]. Lam, N. H. et al., exploited a biophysically-based spiking circuit model consisting of pyramidal excitatory neurons and inhibitory interneurons to study the impact of E/I imbalance on decision-making, an important aspect of cognition [1]. They suggested that both elevating or lowering the E/I ratio impaired cognitive function [1]. Decision-making was impulsive when increasing the E/I ratio (by enhancing excitatory connections), and, when the E/I ratio was lowered (by enhancing inhibitory connections), decision-making was indecisive [1]. These findings set the stage for another important discussion, whether heightened excitation, in a way that elevates the E/I ratio to an abnormal degree, could signify functional impairments in MS. This is particularly important in MS since hyperexcitability has been linked to disease activity [17].

Another interesting result from our study builds on previous findings demonstrating sex-specific relationships between brain

excitability assessed with TMS and cognitive function in MS participants [16]. Specifically, we found that TMS-assessed ipsilateral silent period onset, a marker of transcallosal communication speed, correlated with cognitive function in males only, particularly when assessed in the stronger hand, i.e., when TMS was delivered to the hemisphere corresponding to the weaker hand [16]. Our current findings similarly suggest sex differences in the relationship between E/I ratio and cognitive function (SDMT). We observed that while females showed a relationship between E/I ratio measured in the hemisphere corresponding to the weaker hand, males exhibited this relationship in the opposite hemisphere, i.e., the hemisphere corresponding to the stronger hand. These findings suggest varying lateralization patterns between males and females with MS, highlighting the need for further research in this area.

4.3. Convergent and divergent findings on the link between cortical excitability and MS-related disability

In the MS model, upregulation of pro-inflammatory cytokines leads to hyperexcitability and excitotoxicity-related neuronal damage [68]. Blockage of the a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor reduced excitotoxic neurodegeneration and mitigated the severity of MS; excitotoxic damage has been similarly linked to GABAergic activity [68–72]. Similarly, in people with MS, higher cerebral spinal fluid concentrations of pro-inflammatory cytokines (RANTES, IL-1β) were associated with enhanced intracortical facilitation, reduced short intracortical inhibition, and shortened CSP [21,24]. Enhanced intracortical facilitation and shortened CSP in people with MS experiencing a relapse [24,25] emphasizes that increased neuroinflammation causes hyperexcitability. These results raise important questions regarding whether non-invasive brain stimulation should be used to excite brains that are perhaps already hyperexcitable. However, whilst hyperexcitability (i.e., enhanced excitation and reduced inhibition) is associated with worse outcomes in experimental models, this is not the case in people with MS. For example, Nantes et al. reported prolonged CSP in people with MS-related upper extremity impairment [19]. Mori, F. et al. showed an association between disability and reduced short-intracortical facilitation [23]. These findings concur with our previous work reporting associations between reduced excitation and increased inhibition with higher disability and MS-related symptoms (pain, fatigue, motor and cognitive impairments) [15,33], and higher excitability at earlier MS stages [21,68,73], followed by a shift to lower excitability (from hyperexcitability to hypoexcitability) as the disease progresses [18]. The present study further contributes by demonstrating a relationship between lower E/I ratios and disability and poorer performance. Other TMS studies have shown enhancement of cortical excitability and neuroplastic capacity in people with progressive MS following rehabilitation. For example, in progressive MS, 4-weeks of daily D-Aspartate supplementation enhanced intracortical facilitation and restored neuroplastic capacity [74]. We have previously published an association between lower levels of cardiorespiratory fitness and higher fatigue with prolonged CSP in people with MS [75]. In progressive MS, we noticed enhanced excitability (reductions in AMT and shortening of CSP) following 3 months of body-weight supported treadmill exercise training [34]. Therefore, while most studies in MS models demonstrate that experimentally silencing cortical excitability and increasing inhibition can mitigate MS symptoms, the TMS literature indicates the opposite; enhancing cortical excitability is likely protective against MS and can mitigate symptoms [76–78].

4.4. Implications for future research investigating neuromodulation for MS

Our endeavor to uncover the circuitry (excitation and inhibition) that most likely influences E/I is relevant because of the numerous therapies, particularly, non-invasive brain stimulation techniques, that can induce distinct effects on cortical excitability. While most methods of excitation-inducing brain stimulation (e.g., repetitive TMS, theta burst stimulation, transcranial direct current stimulation) increase cortical excitation and reduce inhibition [79], other approaches, such as quadri-pulse stimulation, act solely on increasing cortical excitation, with no effects on inhibition [80]. Our group previously reported that higher cardiorespiratory fitness was associated with shortened CSP in people with MS, but not with TMS variables indexing excitation [75]. We also noticed shortening of CSP with no changes in MEP amplitudes after longer-term exercise training in people with progressive MS [34]. This effect, however, was noticed in the hemisphere contralateral to the stronger body side, likely more intact and with preserved capacity to undergo therapy-induced neuroplasticity [34]. We suggest that, in people with MS with accumulated disability and those who present with lower E/I ratios, treatment strategies should prioritize excitation-inducing protocols. In the case of brain stimulation protocols and similar approaches where typically one hemisphere is treated, the most affected brain hemisphere should be prioritized. Despite the cross-sectional TMS studies demonstrating associations between enhanced excitability and lower disability, the findings from experimental models reporting excitotoxicity in MS cannot be ignored [68,71,72,81]. Presently, it is unknown whether the abnormally enhanced excitability, typical of early MS, is a compensatory mechanism that serves to maintain function through enhanced neuroplasticity [14,82], or is a factor that, in the long term, contributes to disease progression due to excitotoxicity-related neuronal damage [81]. Caution should be taken when introducing excitatory-inducing protocols in early MS, and more research addressing this topic is necessary.

4.5. Limitations and strengths

This study is not without its limitations. First, the absence of matched controls as well as the lack of longitudinal examination restricts our understanding of the findings. Additionally, our cohort did not include participants with disability levels exceeding EDSS 6.5, limiting the representation of the entire MS disability spectrum. Future research should address these limitations with protocols that can be administered across the disability spectrum. Strengths should also be acknowledged. In addition to a relatively large sample size, one notable strength is the consecutive recruitment of participants from a specialized MS clinic, capturing a diverse and comprehensive representation of the local MS population. Furthermore, the use of a single experimenter for TMS data collection

minimizes inter-rater variability and ensures assessment consistency. Also, rather than TMS stimulation at a fixed intensity, the currently employed recruitment curve experiment was adjusted based on individual motor thresholds for a more accurate assessment of MEPs and CSPs across participants. Finally, the integration of a neuronavigation device in our TMS assessment guaranteed precise targeting of the primary motor cortex and provided guidance of the TMS coil during experiments, reducing subjectivity and enhancing reliability. Furthermore, this study offers a cost-effective and simplified method for analyzing E/I (im)balance compared to conventional methods like MRI, warranting further exploration in future trials. Additionally, the biomarker's ability to predict functionality beyond motor areas, such as cognitive function, suggests its potential utility in broader functional studies. Subsequent investigations should examine its efficacy in such contexts.

5. Conclusion

This study investigated E/I (im)balance using TMS in individuals with MS across disability levels. We report three key observations. Firstly, individuals with higher disability exhibited lower TMS-probed E/I ratios compared to those with lower disability. This is due to decreased overall excitation and increased overall inhibition, particularly in the hemisphere corresponding to the weaker body side. Secondly, the TMS-probed E/I ratio predicted disability status, upper extremity dexterity and cognitive performance, and walking speed, even after controlling for demographic and clinical factors. Additionally, the E/I ratio demonstrated 'excellent' discriminatory ability between high and low disability levels, highlighting its potential as a biomarker in MS. Importantly, this study confirmed the validity of analyzing TMS-induced MEP and their corresponding CSP as a means to assess E/I (im)balance. Lastly, the results indicated a stronger correlation between overall excitation and the E/I ratio compared to overall inhibition, suggesting a potentially greater role of excitation in the E/I imbalance observed in MS. Collectively, these findings support the notion that targeted interventions aimed at increasing cortical excitation and/or reducing inhibition could restore E/I balance, thus holding promise for reducing disability or stalling progression in individuals with MS.

Funding

M.P. received funding from The Canadian Institutes for Health Research [Grant #s: 169649, 173526], Newfoundland and Labrador Research and Development Corporation [Grant #: 5404.1699.104], and Canada Foundation for Innovation [Grant #: 33621]. M.P. is currently receiving funding from the Canada Research Chairs Program [Grant #: 230457]. A.C. received funding from the O'Dea Family Fellowship and is currently receiving funding from the uOttawa/CHEO Research Institute Joint Physical Activity and Health Initiative Postdoctoral Fellowship, Trainee Research in Multiple Sclerosis (University of Ottawa), Multiple Sclerosis Society of Canada endMS Post-Doctoral Fellowship.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Arthur R. Chaves: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sara Tremblay:** Writing – review & editing, Supervision. **Lara Pilutti:** Writing – review & editing, Supervision, Software. **Michelle Ploughman:** Writing – review & editing, Supervision, Software, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Michelle Ploughman reports financial support was provided by The Canadian Institutes for Health Research. Michelle Ploughman reports financial support was provided by Newfoundland and Labrador Research and Development Corporation. Michelle Ploughman reports financial support was provided by Canada Foundation for Innovation. Michelle Ploughman reports financial support was provided by Canada Research Chairs Program. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35834.

References

[1] N.H. Lam, T. Borduqui, J. Hallak, A. Roque, A. Anticevic, J.H. Krystal, X.J. Wang, J.D. Murray, Effects of altered excitation-inhibition balance on decision making in a cortical circuit model, J. Neurosci. : the official journal of the Society for Neuroscience 42 (2022) 1035–1053.

[2] I. Bernardino, A. Dionísio, I.R. Violante, R. Monteiro, M. Castelo-Branco, Motor cortex excitation/inhibition imbalance in young adults with autism spectrum disorder: a MRS-TMS approach, Front. Psychiatr. 13 (2022) 860448.

[3] O.L. Lopatina, N.A. Malinovskaya, Y.K. Komleva, Y.V. Gorina, A.N. Shuvaev, R.Y. Olovyannikova, O.S. Belozor, O.A. Belova, H. Higashida, A.B. Salmina, Excitation/inhibition imbalance and impaired neurogenesis in neurodevelopmental and neurodegenerative disorders, Rev. Neurosci. 30 (2019) 807–820.

[4] E. Lee, J. Lee, E. Kim, Excitation/inhibition imbalance in animal models of autism spectrum disorders, Biol. Psychiatr. 81 (2017) 838–847.

[5] O. Yizhar, L.E. Fenno, M. Prigge, F. Schneider, T.J. Davidson, D.J. O'Shea, V.S. Sohal, I. Goshen, J. Finkelstein, J.T. Paz, K. Stehfest, R. Fudim, C. Ramakrishnan, J.R. Huguenard, P. Hegemann, K. Deisseroth, Neocortical excitation/inhibition balance in information processing and social dysfunction, Nature 477 (2011) 171–178.

[6] H.-T. Chao, H. Chen, R.C. Samaco, M. Xue, M. Chahrour, J. Yoo, J.L. Neul, S. Gong, H.-C. Lu, N. Heintz, M. Ekker, J.L.R. Rubenstein, J.L. Noebels, C. Rosenmund, H.Y. Zoghbi, Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes, Nature 468 (2010) 263–269.

[7] L.E. Potter, J.W. Paylor, J.S. Suh, G. Tenorio, J. Caliaperumal, F. Colbourne, G. Baker, I. Winship, B.J. Kerr, Altered excitatory-inhibitory balance within somatosensory cortex is associated with enhanced plasticity and pain sensitivity in a mouse model of multiple sclerosis, J. Neuroinflammation 13 (2016) 142.

[8] J.H. Foss-Feig, B.D. Adkinson, J.L. Ji, G. Yang, V.H. Srihari, J.C. McPartland, J.H. Krystal, J.D. Murray, A. Anticevic, Searching for cross-diagnostic convergence: neural mechanisms governing excitation and inhibition balance in Schizophrenia and autism spectrum disorders, Biol. Psychiatr. 81 (2017) 848–861.

[9] M.J. Mansilla, S. Presas-Rodríguez, A. Teniente-Serra, I. González-Larreategui, B. Quirant-Sánchez, F. Fondelli, N. Djedovic, D. Iwaszkiewicz-Grześ, K. Chwojnicki, Đ. Miljković, P. Trzonkowski, C. Ramo-Tello, E.M. Martínez-Cáceres, Paving the way towards an effective treatment for multiple sclerosis: advances in cell therapy, Cell. Mol. Immunol. 18 (2021) 1353–1374.

[10] B.D. Trapp, M. Vignos, J. Dudman, A. Chang, E. Fisher, S.M. Staugaitis, H. Battapady, S. Mork, D. Ontaneda, S.E. Jones, R.J. Fox, J. Chen, K. Nakamura, R. A. Rudick, Cortical neuronal densities and cerebral white matter demyelination in multiple sclerosis: a retrospective study, Lancet Neurol. 17 (2018) 870–884.

[11] A. Compston, A. Coles, Multiple sclerosis, Lancet 372 (2008) 1502–1517.

[12] F.D. Lublin, S.C. Reingold, J.A. Cohen, G.R. Cutter, P.S. Sørensen, A.J. Thompson, J.S. Wolinsky, L.J. Balcer, B. Banwell, F. Barkhof, B. Bebo Jr., P.A. Calabresi, M. Clanet, G. Comi, R.J. Fox, M.S. Freedman, A.D. Goodman, M. Inglese, L. Kappos, B.C. Kieseier, J.A. Lincoln, C. Lubetzki, A.E. Miller, X. Montalban, P. W. O'Connor, J. Petkau, C. Pozzilli, R.A. Rudick, M.P. Sormani, O. Stüve, E. Waubant, C.H. Polman, Defining the clinical course of multiple sclerosis: the 2013 revisions, Neurology 83 (2014) 278–286.

[13] L.K. Fisniku, P.A. Brex, D.R. Altmann, K.A. Miszkiel, C.E. Benton, R. Lanyon, A.J. Thompson, D.H. Miller, Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis, Brain : J. Neurol. 131 (2008) 808–817.

[14] C. Balloff, I.-K. Penner, M. Ma, I. Georgiades, L. Scala, N. Troullinakis, J. Graf, D. Kremer, O. Aktas, H.-P. Hartung, S.G. Meuth, A. Schnitzler, S.J. Groiss, P. Albrecht, The degree of cortical plasticity correlates with cognitive performance in patients with Multiple Sclerosis, Brain Stimul. 15 (2022) 403–413.

[15] A.R. Chaves, N.J. Snow, L.R. Alcock, M. Ploughman, Probing the brain–body connection using transcranial magnetic stimulation (TMS): validating a promising tool to provide biomarkers of neuroplasticity and central nervous system function, Brain Sci. 11 (2021) 384.

[16] A.R. Chaves, H.M. Kenny, N.J. Snow, R.W. Pretty, M. Ploughman, Sex-specific disruption in corticospinal excitability and hemispheric (a)symmetry in multiple sclerosis, Brain Res. 1773 (2021) 147687.

[17] M. Stampanoni Bassi, F. Buttari, L. Gilio, N. De Paolis, D. Fresegna, D. Centonze, E. Iezzi, Inflammation and corticospinal functioning in multiple sclerosis: a TMS perspective, Front. Neurol. 11 (2020) 566.

[18] A.R. Chaves, E.M. Wallack, L.P. Kelly, R.W. Pretty, H.D. Wiseman, A. Chen, C.S. Moore, M. Stefanelli, M. Ploughman, Asymmetry of brain excitability: a new biomarker that predicts objective and subjective symptoms in multiple sclerosis, Behav. Brain Res. 359 (2019) 281–291.

[19] J.C. Nantes, J. Zhong, S.A. Holmes, B. Whatley, S. Narayanan, Y. Lapierre, D.L. Arnold, L. Koski, Intracortical inhibition abnormality during the remission phase of multiple sclerosis is related to upper limb dexterity and lesions, Clin. Neurophysiol. : official journal of the International Federation of Clinical Neurophysiology 127 (2016) 1503–1511.

[20] J.C. Nantes, J. Zhong, S.A. Holmes, S. Narayanan, Y. Lapierre, L. Koski, Cortical damage and disability in multiple sclerosis: relation to intracortical inhibition and facilitation, Brain Stimul. 9 (2016) 566–573.

[21] F. Mori, R. Nisticò, C.G. Nicoletti, S. Zagaglia, G. Mandolesi, S. Piccinin, G. Martino, A. Finardi, P.M. Rossini, G.A. Marfia, R. Furlan, D. Centonze, RANTES correlates with inflammatory activity and synaptic excitability in multiple sclerosis, Mult. Scler. 22 (2016) 1405–1412.

[22] S.S. Ayache, A. Créange, W.H. Farhat, H.G. Zouari, C. Lesage, U. Palm, M. Abdellaoui, J.P. Lefaucheur, Cortical excitability changes over time in progressive multiple sclerosis, Funct. Neurol. 30 (2015) 257–263.

[23] F. Mori, H. Kusayanagi, F. Monteleone, A. Moscatelli, C.G. Nicoletti, G. Bernardi, D. Centonze, Short interval intracortical facilitation correlates with the degree of disability in multiple sclerosis, Brain Stimul. 6 (2013) 67–71.

[24] S. Rossi, R. Furlan, V. De Chiara, C. Motta, V. Studer, F. Mori, A. Musella, A. Bergami, L. Muzio, G. Bernardi, L. Battistini, G. Martino, D. Centonze, Interleukin-1β causes synaptic hyperexcitability in multiple sclerosis, Ann. Neurol. 71 (2012) 76–83.

[25] M.D. Caramia, M.G. Palmieri, M.T. Desiato, L. Boffa, P. Galizia, P.M. Rossini, D. Centonze, G. Bernardi, Brain excitability changes in the relapsing and remitting phases of multiple sclerosis: a study with transcranial magnetic stimulation, Clin. Neurophysiol. : official journal of the International Federation of Clinical Neurophysiology 115 (2004) 956–965.

[26] D.H. Mahad, B.D. Trapp, H. Lassmann, Pathological mechanisms in progressive multiple sclerosis, the Lancet, Neurology 14 (2015) 183–193.

[27] R. Dutta, B.D. Trapp, Relapsing and progressive forms of multiple sclerosis: insights from pathology, Curr. Opin. Neurol. 27 (2014) 271–278.

[28] N.J. Snow, K.P. Wadden, A.R. Chaves, M. Ploughman, Transcranial magnetic stimulation as a potential biomarker in multiple sclerosis: a systematic review with recommendations for future research, Neural Plast. 2019 (2019) 6430596.

[29] P.M. Rossini, D. Burke, R. Chen, L.G. Cohen, Z. Daskalakis, R. Di Iorio, V. Di Lazzaro, F. Ferreri, P.B. Fitzgerald, M.S. George, M. Hallett, J.P. Lefaucheur, B. Langguth, H. Matsumoto, C. Miniussi, M.A. Nitsche, A. Pascual-Leone, W. Paulus, S. Rossi, J.C. Rothwell, H.R. Siebner, Y. Ugawa, V. Walsh, U. Ziemann, 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. : official journal of the International Federation of Clinical Neurophysiology 126 (2015) 1071–1107.

[30] B. Bielekova, R. Martin, Development of biomarkers in multiple sclerosis, Brain : J. Neurol. 127 (2004) 1463–1478.

[31] M. Orth, J.C. Rothwell, The cortical silent period: intrinsic variability and relation to the waveform of the transcranial magnetic stimulation pulse, Clin. Neurophysiol. : official journal of the International Federation of Clinical Neurophysiology 115 (2004) 1076–1082.

[32] C.M. Epstein, E.M. Wassermann, U. Ziemann, A. Wolters, U. Ziemann, R. Benecke, The Cortical Silent Period, Oxford University Press, 2012.

[33] H.M. Murphy, C.M. Fetter, N.J. Snow, A.R. Chaves, M.B. Downer, M. Ploughman, Lower corticospinal excitability and greater fatigue among people with multiple sclerosis experiencing pain, Multiple sclerosis journal - experimental, translational and clinical 9 (2023) 20552173221143398.

[34] A.R. Chaves, A.J. Devasahayam, M. Riemenschneider, R.W. Pretty, M. Ploughman, Walking training enhances corticospinal excitability in progressive multiple sclerosis—a pilot study, Front. Neurol. 11 (2020).

[35] A.R. Chaves, A.J. Devasahayam, L.P. Kelly, R.W. Pretty, M. Ploughman, Exercise-induced brain excitability changes in progressive multiple sclerosis: a pilot study, J. Neurol. Phys. Ther. : J. Neurol. Phys. Ther. 44 (2020) 132–144.

[36] A.J. Thompson, B.L. Banwell, F. Barkhof, W.M. Carroll, T. Coetzee, G. Comi, J. Correale, F. Fazekas, M. Filippi, M.S. Freedman, K. Fujihara, S.L. Galetta, H. P. Hartung, L. Kappos, F.D. Lublin, R.A. Marrie, A.E. Miller, D.H. Miller, X. Montalban, E.M. Mowry, P.S. Sorensen, M. Tintore, A.L. Traboulsee, M. Trojano, B.M. J. Uitdehaag, S. Vukusic, E. Waubant, B.G. Weinshenker, S.C. Reingold, J.A. Cohen, Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria, Lancet Neurol. 17 (2018) 162–173.

[37] S. Rossi, M. Hallett, P.M. Rossini, A. Pascual-Leone, T.M.S.C.G. Safety of, Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research, Clin. Neurophysiol. : official journal of the International Federation of Clinical Neurophysiology 120 (2009) 2008–2039.
[38] J.P. Vandenbroucke, E. von Elm, D.G. Altman, P.C. Gøtzsche, C.D. Mulrow, S.J. Pocock, C. Poole, J.J. Schlesselman, M. Egger, S.I. for the, Strengthening the reporting of observational studies in Epidemiology (STROBE): explanation and elaboration, PLoS Med. 4 (2007) e297.
[39] J.F. Kurtzke, Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS), Neurology 33 (1983) 1444–1452.
[40] A.S. Drake, B. Weinstock-Guttman, S.A. Morrow, D. Hojnacki, F.E. Munschauer, R.H. Benedict, Psychometrics and normative data for the multiple sclerosis functional composite: replacing the PASAT with the symbol digit modalities test, Mult. Scler. 16 (2010) 228–237.
[41] R.H. Benedict, M.K. Walton, Evaluating cognitive outcome measures for MS clinical trials: what is a clinically meaningful change? Mult. Scler. 18 (2012) 1673–1679.
[42] P. Feys, I. Lamers, G. Francis, R. Benedict, G. Phillips, N. LaRocca, L.D. Hudson, R. Rudick, The Nine-Hole Peg Test as a manual dexterity performance measure for multiple sclerosis, Mult. Scler. 23 (2017) 711–720.
[43] V. Mathiowetz, K. Weber, N. Kashman, G. Volland, Adult norms for the nine hole peg test of finger dexterity, the occupational therapy, J. Res. 5 (1985) 24–38.
[44] R.W. Bohannon, Comfortable and maximum walking speed of adults aged 20-79 years: reference values and determinants, Age Ageing 26 (1997) 15–19.
[45] N.E. Fritz, J. Keller, P.A. Calabresi, K.M. Zackowski, Quantitative measures of walking and strength provide insight into brain corticospinal tract pathology in multiple sclerosis, Neuroimage: Clinical 14 (2017) 490–498.
[46] H. Bruining, R. Hardstone, E.L. Juarez-Martinez, J. Sprengers, A.-E. Avramiea, S. Simpraga, S.J. Houtman, S.-S. Poil, E. Dallares, S. Palva, B. Oranje, J. Matias Palva, H.D. Mansvelder, K. Linkenkaer-Hansen, Measurement of excitation-inhibition ratio in autism spectrum disorder using critical brain dynamics, Sci. Rep. 10 (2020) 9195.
[47] H. Hotelling, The generalization of student's ratio, Ann. Math. Stat. 2 (1931) 360–378, 319.
[48] K.A. Pituch, J.P. Stevens, Applied Multivariate Statistics for the Social Sciences: Analyses with SAS and IBM's SPSS, sixth ed. (sixth ed., 2015.
[49] C. Tataroglu, A. Genc, E. Idiman, R. Cakmur, F. Idiman, Cortical silent period and motor evoked potentials in patients with multiple sclerosis, Clin. Neurol. Neurosurg. 105 (2003) 105–110.
[50] L.M. Jorgensen, J.E. Nielsen, M. Ravnborg, MEP recruitment curves in multiple sclerosis and hereditary spastic paraplegia, J. Neurol. Sci. 237 (2005) 25–29.
[51] G.W. Thickbroom, M.L. Byrnes, S.A. Archer, A.G. Kermode, F.L. Mastaglia, Corticomotor organisation and motor function in multiple sclerosis, J. Neurol. 252 (2005) 765–771.
[52] A. Gagliardo, F. Galli, A. Grippo, A. Amantini, C. Martinelli, M.P. Amato, W. Borsini, Motor evoked potentials in multiple sclerosis patients without walking limitation: amplitude vs. conduction time abnormalities, J. Neurol. 254 (2007) 220–227.
[53] J.L. Neva, B. Lakhani, K.E. Brown, K.P. Wadden, C.S. Mang, N.H. Ledwell, M.R. Borich, I.M. Vavasour, C. Laule, A.L. Traboulsee, A.L. MacKay, L.A. Boyd, Multiple measures of corticospinal excitability are associated with clinical features of multiple sclerosis, Behav. Brain Res. 297 (2016) 187–195.
[54] S.S. Ayache, A. Creange, W.H. Farhat, H.G. Zouari, V. Mylius, R. Ahdab, M. Abdellaoui, J.P. Lefaucheur, Relapses in multiple sclerosis: effects of high-dose steroids on cortical excitability, Eur. J. Neurol. : the official journal of the European Federation of Neurological Societies 21 (2014) 630–636.
[55] S.S. Ayache, A. Creange, W.H. Farhat, H.G. Zouari, C. Lesage, U. Palm, M. Abdellaoui, J.P. Lefaucheur, Cortical excitability changes over time in progressive multiple sclerosis, Funct. Neurol. 30 (2015) 257–263.
[56] D. Zeller, K. aufm Kampe, A. Biller, K. Stefan, R. Gentner, A. Schutz, A. Bartsch, M. Bendszus, K.V. Toyka, P. Rieckmann, J. Classen, Rapid-onset central motor plasticity in multiple sclerosis, Neurology 74 (2010) 728–735.
[57] N. Kale, J. Agaoglu, G. Onder, O. Tanik, Correlation between disability and transcranial magnetic stimulation abnormalities in patients with multiple sclerosis, J. Clin. Neurosci. : official journal of the Neurosurgical Society of Australasia 16 (2009) 1439–1442.
[58] N. Kale, J. Agaoglu, O. Tanik, Electrophysiological and clinical correlates of corpus callosum atrophy in patients with multiple sclerosis, Neurol. Res. 32 (2010) 886–890.
[59] C.M. Stinear, J.P. Coxon, W.D. Byblow, Primary motor cortex and movement prevention: where Stop meets Go, Neurosci. Biobehav. Rev. 33 (2009) 662–673.
[60] A. Priori, A. Berardelli, M. Inghilleri, L. Polidori, M. Manfredi, Electromyographic silent period after transcranial brain stimulation in Huntington's disease, Mov. Disord. : official journal of the Movement Disorder Society 9 (1994) 178–182.
[61] E.M. Khedr, M.A. Ahmed, A.M. Ali, R. Badry, J.C. Rothwell, Changes in motor cortical excitability in patients with Sydenham's chorea, Mov. Disord. : official journal of the Movement Disorder Society 30 (2015) 259–262.
[62] J. Classen, A. Schnitzler, F. Binkofski, K.J. Werhahn, Y.S. Kim, K.R. Kessler, R. Benecke, The motor syndrome associated with exaggerated inhibition within the primary motor cortex of patients with hemiparetic, Brain : J. Neurol. 120 (Pt 4) (1997) 605–619.
[63] H.J. Braune, C. Fritz, Transcranial magnetic stimulation-evoked inhibition of voluntary muscle activity (silent period) is impaired in patients with ischemic hemispheric lesion, Stroke; a journal of cerebral circulation 26 (1995) 550–553.
[64] J. DeLuca, N.D. Chiaravalloti, B.M. Sandroff, Treatment and management of cognitive dysfunction in patients with multiple sclerosis, Nat. Rev. Neurol. 16 (2020) 319–332.
[65] G.C. DeLuca, R.L. Yates, H. Beale, S.A. Morrow, Cognitive impairment in multiple sclerosis: clinical, radiologic and pathologic insights, Brain Pathol. 25 (2015) 79–98.
[66] F. Akbarian, C. Rossi, L. Costers, M.B. D'hooghe, M. D'haeseleer, G. Nagels, J. Van Schependom, The spectral slope as a marker of excitation/inhibition ratio and cognitive functioning in multiple sclerosis, bioRxiv 2023.2001.2023 (2023), 525139.
[67] O. Yizhar, L.E. Fenno, M. Prigge, F. Schneider, T.J. Davidson, D.J. O'Shea, V.S. Sohal, I. Goshen, J. Finkelstein, J.T. Paz, K. Stehfest, R. Fudim, C. Ramakrishnan, J.R. Huguenard, P. Hegemann, K. Deisseroth, Neocortical excitation/inhibition balance in information processing and social dysfunction, Nature 477 (2011) 171–178.
[68] D. Centonze, L. Muzio, S. Rossi, F. Cavasinni, V. De Chiara, A. Bergami, A. Musella, M. D'Amelio, V. Cavallucci, A. Martorana, A. Bergamaschi, M.T. Cencioni, A. Diamantini, E. Butti, G. Comi, G. Bernardi, F. Cecconi, L. Battistini, R. Furlan, G. Martino, Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis, J. Neurosci. : the official journal of the Society for Neuroscience 29 (2009) 3442–3452.
[69] D. Stellwagen, E.C. Beattie, J.Y. Seo, R.C. Malenka, Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha, J. Neurosci. : the official journal of the Society for Neuroscience 25 (2005) 3219–3228.
[70] H. Pribiag, D. Stellwagen, TNF-α downregulates inhibitory neurotransmission through protein phosphatase 1-dependent trafficking of GABA(A) receptors, J. Neurosci. : the official journal of the Society for Neuroscience 33 (2013) 15879–15893.
[71] S. Rossi, L. Muzio, V. De Chiara, G. Grasselli, A. Musella, G. Musumeci, G. Mandolesi, R. De Ceglia, S. Maida, E. Biffi, A. Pedrocchi, A. Menegon, G. Bernardi, R. Furlan, G. Martino, D. Centonze, Impaired striatal GABA transmission in experimental autoimmune encephalomyelitis, Brain Behav. Immun. 25 (2011) 947–956.
[72] R. Bhat, R. Axtell, A. Mitra, M. Miranda, C. Lock, R.W. Tsien, L. Steinman, Inhibitory Role for GABA in Autoimmune Inflammation, vol. 107, Proceedings of the National Academy of Sciences, 2010, pp. 2580–2585.
[73] M. Stampanoni Bassi, F. Mori, F. Buttari, G.A. Marfia, A. Sancesario, D. Centonze, E. Iezzi, Neurophysiology of synaptic functioning in multiple sclerosis, Clin. Neurophysiol. : official journal of the International Federation of Clinical Neurophysiology 128 (2017) 1148–1157.
[74] C.G. Nicoletti, F. Monteleone, G.A. Marfia, A. Usiello, F. Buttari, D. Centonze, F. Mori, Oral D-Aspartate enhances synaptic plasticity reserve in progressive multiple sclerosis, Mult. Scler. 26 (2020) 304–311.
[75] A.R. Chaves, L.P. Kelly, C.S. Moore, M. Stefanelli, M. Ploughman, Prolonged cortical silent period is related to poor fitness and fatigue, but not tumor necrosis factor, in: Multiple Sclerosis, Clinical Neurophysiology, 2019.
[76] X. Zhang, Y. Huai, Z. Wei, W. Yang, Q. Xie, L. Yi, Non-invasive brain stimulation therapy on neurological symptoms in patients with multiple sclerosis: a network meta analysis, Front. Neurol. 13 (2022).

[77] W.-Y. Hsu, C.-H. Cheng, T.P. Zanto, A. Gazzaley, R.M. Bove, Effects of transcranial direct current stimulation on cognition, mood, pain, and fatigue in multiple sclerosis: a systematic review and meta-analysis, Front. Neurol. 12 (2021).
[78] X. Chen, L. Yin, Y. An, H. Yan, T. Zhang, X. Lu, J. Yan, Effects of repetitive transcranial magnetic stimulation in multiple sclerosis: a systematic review and meta-analysis, Multiple sclerosis and related disorders 59 (2022) 103564.
[79] P.B. Fitzgerald, S. Fountain, Z.J. Daskalakis, A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition, Clin. Neurophysiol. 117 (2006) 2584–2596.
[80] M. Hamada, Y. Terao, R. Hanajima, Y. Shirota, S. Nakatani-Enomoto, T. Furubayashi, H. Matsumoto, Y. Ugawa, Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation, J. Physiol. 586 (2008) 3927–3947.
[81] D. Pitt, P. Werner, C.S. Raine, Glutamate excitotoxicity in a model of multiple sclerosis, Nat. Med. 6 (2000) 67–70.
[82] V. Tomassini, P.M. Matthews, A.J. Thompson, D. Fuglø, J.J. Geurts, H. Johansen-Berg, D.K. Jones, M.A. Rocca, R.G. Wise, F. Barkhof, J. Palace, Neuroplasticity and functional recovery in multiple sclerosis, Nature reviews, Neurology 8 (2012) 635–646.