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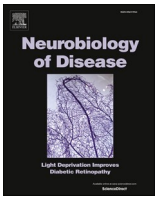

Neural bases of motor fatigue in multiple sclerosis: A multimodal approach using neuromuscular assessment and TMS-EEG

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ABSTRACT

Motor fatigue is one of the most common symptoms in multiple sclerosis (MS) patients. Previous studies suggested that increased motor fatigue in MS may arise at the central nervous system level. However, the mechanisms underlying central motor fatigue in MS are still unclear.

This paper investigated whether central motor fatigue in MS reflects impaired corticospinal transmission or suboptimal primary motor cortex (M1) output (supraspinal fatigue). Furthermore, we sought to identify whether central motor fatigue is associated with abnormal M1 excitability and connectivity within the sensorimotor network.

Twenty-two patients affected by relapsing-remitting MS and 15 healthy controls (HCs) performed repeated blocks of contraction at different percentages of maximal voluntary contraction with the right first dorsal interosseus muscle until exhaustion. Peripheral, central, and supraspinal components of motor fatigue were quantified by a neuromuscular assessment based on the superimposed twitch evoked by peripheral nerve and transcranial magnetic stimulation (TMS). Corticospinal transmission, excitability and inhibition during the task were tested by measurement of motor evoked potential (MEP) latency, amplitude, and cortical silent period (CSP). M1 excitability and connectivity was measured by TMS-evoked electroencephalography (EEG) potentials (TEPs) elicited by M1 stimulation before and after the task.

Patients completed fewer blocks of contraction and showed higher values of central and supraspinal fatigue than HCs. We found no MEP or CSP differences between MS patients and HCs. Patients showed a post-fatigue increase in TEPs propagation from M1 to the rest of the cortex and in source-reconstructed activity within the sensorimotor network, in contrast to the reduction observed in HCs. Post-fatigue increase in source-reconstructed TEPs correlated with supraspinal fatigue values.

To conclude, MS-related motor fatigue is caused by central mechanisms related explicitly to suboptimal M1 output rather than impaired corticospinal transmission. Furthermore, by adopting a TMS-EEG approach, we proved that suboptimal M1 output in MS patients is associated with abnormal task-related modulation of M1 connectivity within the sensorimotor network. Our findings shed new light on the central mechanisms of motor fatigue in MS by highlighting a possible role of abnormal sensorimotor network dynamics. These novel results may point to new therapeutical targets for fatigue in MS.

Abbreviations: multiple sclerosis, MS; primary motor cortex, M1; healthy controls, HCs; transcranial magnetic stimulation, TMS; motor evoked potential, MEP; cortical silent period, CSP; TMS-evoked potentials, TEPs; Beck Depression Inventory-II, BDI-II; Expanded Disability Status Scale, EDSS; Modified Fatigue Impact Scale, MFIS; Fatigue Scale for Motor and Cognitive Functions, FSMC; Fatigue Symptoms and Impacts Questionnaire – Relapsing Multiple Sclerosis, FSIQ; first dorsal interosseous, FDI; peripheral nerve stimulation, PNS; Electromyography, EMG; maximal voluntary contraction, MVC; superimposed twitch, SIT; PNS-evoked superimposed twitch, PNS-SIT; post-twitch, PT; TMS-evoked superimposed twitch, TMS-SIT; resting motor threshold, RMT; local mean field amplitude, LMFA; global mean field power, GMFP; analysis of variance, ANOVA; region of interest, ROI; time window of interest, TOI; Motor fatigue index, MFI.

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1. Introduction

Motor fatigue is a common symptom reported by patients with Multiple Sclerosis (MS)(Braley and Chervin, 2010; Chalah et al., 2015) and can significantly affect their ability to perform everyday tasks. (Kluger et al., 2013) Motor fatigue, an exercise-induced decline in a muscle's force during sustained activity, involves central and peripheral components that can be assessed using neurophysiological techniques. (Gandevia et al., 1995) In healthy subjects, the central component of motor fatigue results from a reduced corticospinal transmission or suboptimal output from the primary motor cortex (M1), the latter also known as "supraspinal fatigue".(Gandevia, 2001; Gandevia et al., 1996) Previous studies suggested a predominant role of the central component for motor fatigue in relapsing-remitting MS patients whereas the contribution of the peripheral component was considered negligible. (Gandevia, 2001; Steens et al., 2012a; Sheean et al., 1997) In MS, transcranial magnetic stimulation (TMS) studies have suggested that changes in corticospinal excitability and transmission(Coates et al., 2020; Thickbroom et al., 2006) may play a significant role in the pathophysiology of the central component for motor fatigue. However, an impaired output from M1 ("supraspinal fatigue") may also have a pathophysiological role in fatigue. Finally, M1 changes responsible for motor fatigue in MS may be secondary to abnormal sensorimotor network dynamics.(Chalah et al., 2015; Steens et al., 2012a; Fleischer et al., 2022; Morgante et al., 2011; Petajan and White, 2000; Reddy et al., 2000; Rocca et al., 2005; Russo et al., 2017; Specogna et al., 2012) A further investigations of the cortical mechanisms underlying motor fatigue in MS will provide a better understanding of the pathophysiology of fatigue and may lead to novel therapeutical interventions.

Our hypothesis is that motor fatigue in MS patients reflects suboptimal M1 output ("supraspinal fatigue") due to exercise-induced changes in M1 connectivity within the sensorimotor network. Patients with MS complaining of fatigue were asked to perform a fatiguing task with the index finger up until motor exhaustion. To measure the central component of motor fatigue, we adopted a twitch interpolation method throughout the fatiguing task.(Lloyd et al., 1991; Merton, 1954; Thomas et al., 1989) To see whether central fatigue was due to impaired corticospinal transmission or suboptimal M1 output (supraspinal fatigue), we measured MEP latency, amplitude, and cortical silent period (CSP), as well as the superimposed twitch elicited by TMS during the task.(Gandevia et al., 1996; Lee et al., 2008; Taylor and Gandevia, 2001) Finally, to see whether an impaired M1 output during the fatiguing motor task is associated with an abnormal fatigue-induced modulation of M1 excitability and connectivity within the sensorimotor network, we recorded the TMS-evoked potentials (TEPs) elicited by M1 stimulation before and after the motor task. TEPs reflect the excitability of the stimulated cortical area as well as its connectivity with cortical and subcortical areas within its functional network.(Bortoletto et al., 2015; Esposito et al., 2022; Leodori et al., 2022a; Leodori et al., 2020; Ozdemir et al., 2020; Tremblay et al., 2019) The integrative approach characterizing the present study will provide useful insights into the pathophysiology of fatigue in MS.

2. Materials and methods

2.1. Participants

We enrolled 22 patients (37.1 ± 7.2 years, 16 F) complaining of fatigue with a diagnosis of relapsing-remitting MS according to the revised McDonald criteria,(Thompson et al., 2018) and 15 healthy controls (HCs) with similar age and gender distribution (33.5 ± 5.9 years, 10 F). All participants were right-handed as defined by the Edinburgh Handedness Inventory.(Oldfield, 1971) All patients were recruited from MS centers of Sapienza University of Rome (Italy). All participants gave their written informed consent, and the institutional review board approved all study procedures following the Declaration of Helsinki.

Enrolled patients were chronically treated with natalizumab and were clinically and radiologically stable for at least one year. Patients participated in the study procedures between 10 and 7 days before the scheduled natalizumab infusion and none of them had any psychiatric diseases, other neurological conditions, or TMS contraindications.(Rossi et al., 2021) Additional exclusion criteria were severe or moderate depression as determined by a Beck Depression Inventory-II (BDI-II) score > 19,(Quaranta et al., 2012) clinical evidence of weakness, superficial or deep sensory loss, or spasticity in the upper right arm.

Clinical assessment included the Expanded Disability Status Scale (EDSS),(Kurtzke, 1983) the Modified Fatigue Impact Scale (MFIS),(Fisk et al., 1994) the Fatigue Scale for Motor and Cognitive Functions (FSMC),(Penner et al., 2009) the Fatigue Symptoms and Impacts Questionnaire – Relapsing Multiple Sclerosis (FSIQ).(Hudgens et al., 2019) To be enrolled, patients had to have an EDSS score lower than 6.5 and report disabling motor fatigue defined as a ≥ 5 score on the 5-item MFIS version.(Fisk et al., 1994) Clinical characteristics of patients are summarized in Table 1.

2.2. Experimental setup

Subjects were sitting on a chair with their right forearm resting on a padded flat table in the pronated position and the wrist in a neutral position (0° deviation from the forearm), their hand grasping a horizontal handle and their index finger extended and secured to a force transducer. This setup only allowed the recording of index finger abduction force, and contractions were virtually isometric. The hand and wrist were secured to the board by Velcro straps to prevent movement (Fig. 1B top). TMS was performed using a figure-of-eight coil (70-mm diameter) connected to a monophasic stimulator (Magstim, 200 (Chalah et al., 2015)) during the neuromuscular assessment, or a biphasic stimulator (Magstim, Rapid(Chalah et al., 2015)) during TMS-EEG evaluation. The coil was placed over the scalp location that most consistently elicited an MEP in the contralateral first dorsal interosseous (FDI) muscle ('hotspot'), with the handle about 45° away from the midline, in a posterior-anterior direction. The correct positioning of the coil during the experiment was monitored through a neuronavigation system (SofTaxic, EMS) guided by an optical tracking system (Polaris Vicra, Northern Digital Inc., Canada). Using the neuronavigation software, we obtained an estimated individualized MRI for all participants from a mean MR template in the MNI space. Peripheral nerve stimulation (PNS) consisted of square-wave electrical pulses (200-μs duration) delivered using a constant current stimulator (Digitimer DS7AH) with electrodes placed over the right ulnar nerve at the wrist. PNS was delivered as pairs of stimuli separated by 10 ms.

2.3. Data recording

Electromyography (EMG) was recorded using surface Ag/AgCl electrodes over the right FDI muscle, and the force of FDI abduction was recorded with a custom-made handheld force transducer (Fig. 1). (Thickbroom et al., 2006; Szubski et al., 2007) EMG and force signals were amplified (x1000) (D360, Digitimer, UK), sampled at 5 kHz (1401 Plus, CED), and sent to a PC with dedicated software (Signal v.7, CED) for data storage and online visualization. The force exerted by FDI was displayed on a monitor to provide real-time feedback to both examiner and participant.

EEG was recorded with a 32 passive electrode cap (BrainCap, EASYCAP) following the international 10–20 system, bandpass filtered (DC-2.5 kHz), and sampled at 10 kHz with a TMS-compatible DC amplifier (NeurOne, Bittium). We used one additional electrode as a ground (Fpz) and one as a common online reference (POz). Impedance for each electrode was kept below 5 kΩ (Fig. 1C top).

2.4. Baseline measurements

The experimental protocol is summarized in Fig. 1 (Fig. 1A). First, we defined maximal voluntary contraction (MVC) force, PNS, and TMS intensity for each participant. We defined MVC as the mean maximal force produced during 5 s of index finger abduction repeated three times and separated by 60-s resting intervals. PNS intensity was defined as 120% of the minimum intensity needed for the pair of stimuli to elicit its maximal force twitch from the resting FDI.(Steens et al., 2012a; Sheean et al., 1997; Szubski et al., 2007; Steens et al., 2012b) During the third MVC trial, we also delivered PNS during MVC to record the pre-fatigue PNS-evoked superimposed twitch (PNS-SIT), and 2 s after the contraction to record the post-twitch (PT)(Lloyd et al., 1991; Mira et al., 2017) (Fig. 1B middle). In addition, we defined the TMS intensity used in neuromuscular assessment as 110% of the minimum intensity needed to elicit the maximal TMS-evoked superimposed twitch (TMS-SIT) during 5 s of contraction at 50% MVC using single monophasic pulses.(Thomas et al., 2016) Finally, using a biphasic stimulator, we defined the resting motor threshold (RMT) according to standardized procedures(Rossini et al., 1995) (Fig. 1B, bottom).

2.5. Neuromuscular assessment

Fifteen minutes after baseline measurement collection, participants repeated 50-s blocks of index finger abduction at different force levels. Each block consisted of 15 s contraction at 50% of MVC values at baseline, followed immediately by five contractions, each lasting 5 s, respectively at 100%, 87.5%, 75%, 62.5%, and 50% of the MVC recorded in the previous block (MVC in a given block, or MVC'), and then by 10 s of rest.(Mira et al., 2017; Gruet et al., 2014) During each block, PNS was delivered about 2 s after the beginning of the 100% MVC' for central fatigue assessment, and 2 s after the end of contraction for peripheral fatigue assessment.(Merton, 1954; Mira et al., 2017) During each block, TMS single pulses were delivered for each of the 5 MVC' levels for supraspinal fatigue assessment.(Dekerle et al., 2019) TMS pulse during the 100% MVC' was delivered 2 s after PNS (Fig. 1D, top). To match the required force level, participants were given visual feedback of the instantaneous force exerted on a monitor positioned 90 cm in front of the participants' eyes, with horizontal lines updating the target force level online.(Mira et al., 2017) Participants were instructed to reach the required target force after every stimulation as soon as possible. Blocks were repeated until task failure, defined as the inability to maintain a maximal force (i.e., 100% MVC') equal to or >50% of the baseline values (50% MVC) for >2 s (Fig. 1D, bottom).

2.6. TMS-EEG evaluation

One hundred TMS pulses at 90% RMT intensity were delivered during continuous EEG recording before and immediately after the neuromuscular assessment. The interpulse interval randomly varied between 1100 and 1400 ms (Fig. 1C, bottom).(Leodori et al., 2022b) During TMS-EEG evaluation, participants were asked to relax, minimize movements, and keep their eyes open, looking at a fixation point on a screen. During recording, participants wore noise-reduction earmuffs on top of earphones continuously playing a noise designed to mask the TMS click,(Mancuso et al., 2021; Rocchi et al., 2021; Russo et al., 2022) and a thin foam layer was applied beneath the coil to reduce bone conduction and scalp sensation to the TMS pulse (Fig. 1C, middle).

2.7. Data analysis

Data were preprocessed and analyzed using custom scripts in MATLAB (v 2017b).

We computed the motor fatigue index for each participant as the reciprocal of the product of the number of blocks completed before task failure and pre-fatigue MVC. Motor fatigue index values were z-score normalized for further analysis. For each block of neuromuscular assessment, we measured the PNS-SIT recorded during MVC', the PT recorded at post-contraction rest, and the TMS-SIT recorded during each % of MVC. Peripheral fatigue was measured as the progressive decline in PT values, and central fatigue as the progressive increase in PT-normalized PNS-SIT values compared to pre-fatigue values.(Merton, 1954; Thomas et al., 1989; Mira et al., 2017) Supraspinal fatigue values were computed from TMS-SIT values (see supplementary material for peripheral, central and supraspinal computation formulas).(Lee et al., 2008; Mira et al., 2017; Dekerle et al., 2019; Goodall et al., 2009;

Table 1
Patients' demographics and clinical characteristics.

ID	Age (yrs.)	Gender	EDSS	BDI-II	FSMC	MFIS	FSIQ[a]	FSIQ[b]
1	34	F	2.5	15	53	59	58	29
2	35	F	1	0	1	5	27	4
3	20	F	2.5	16	47	38	18	11
4	40	F	1.5	8	58	35	19	26
5	30	F	1.5	16	40	34	58	26
6	37	F	2	14	35	25	24	10
7	37	F	2.5	13	42	35	35	20
8	44	F	4.5	13	44	44	29	31
9	38	F	4	11	56	54	47	34
10	32	M	4	18	65	49	48	26
11	37	F	1.5	18	64	58	54	41
12	50	M	6	15	36	40	49	23
13	37	M	1.5	14	44	36	35	26
14	37	F	0	12	31	12	24	5
15	35	F	1.5	3	6	5	9	2
16	44	M	3	17	58	57	42	27
17	35	F	3	18	14	10	19	0
18	49	M	4.5	9	55	36	45	30
19	37	F	4	18	58	55	50	31
20	27	M	1.5	13	43	36	44	18
21	39	F	1.5	4	17	12	3	6
22	42	F	2.5	18	62	60	24	29
Average (SD)	37.1 (7.2)	F = 16	–	–	–	–	–	–
Median (min – max)	–	–	2.5 (0–6)	14 (0–18)	44 (1–65)	36 (5–60)	35 (3–58)	26 (0–41)

[a] Symptomatic score.
[b] Impact score.

Szubski et al., 2006; Todd et al., 2003) For each block of neuromuscular assessment, we epoched the EMG signal around the TMS stimulus delivered during the 100% MVC' to measure the following variables: the rectified EMG signal averaged across 100 ms pre-TMS (pre-TMS EMG), MEP peak-to-peak amplitude, MEP latency, and CSP duration.(Thickbroom et al., 2006) A detailed description of outcome measure computation is provided in the supplementary materials. All fatigue and TMS-EMG measures from each block of neuromuscular assessment were normalized for the number of blocks completed by each participant by averaging across the first, second, third, and last fourth of blocks to obtain the same number of values in each participant corresponding to 25, 50, 75, and 100% of task failure.

EEG signal recorded in pre- and post-fatigue TMS-EEG blocks was epoched from −1.2 s before to 1.2 s after TMS pulses and preprocessed following a recently described pipeline(Leodori et al., 2022a; Rogasch et al., 2022) (see supplementary material for the step-by-step TMS-EEG preprocessing procedure). After re-referencing to the common average, final TEPs were obtained. The local mean field amplitude (LMFA) was calculated as a measure of local M1 excitability by computing the average of the rectified TEPs from FC1, C3, Cz, and CP1 electrodes. The global mean field power (GMFP) was calculated to characterize the propagation of TMS-evoked activity from M1 to the global scalp. This was calculated by computing the standard deviation (root mean square) across all electrodes at each time point.(Esser et al., 2006) Cleaned TMS-EEG epochs were also imported into Brainstorm(Tadel et al., 2011) (http://neuroimage.usc.edu/brainstorm) to perform source-level reconstruction according to recently described methods and obtain TEP source activation as current density time series at the cortical level. (Ozdemir et al., 2020; Momi et al., 2021) A detailed description of preprocessing steps and TMS-EEG source-level reconstruction is provided in the supplementary materials.

2.8. Statistical analysis

Statistical analysis of demographics, stimulation parameters, fatigue, and TMS-EMG measures was performed using SPSS Statistics (version 25.0.0; IBM). Between-group differences (patients with MS, HCs) regarding gender frequency were investigated using chi-square testing for homogeneity; in contrast, differences in age, stimulation intensity, pre-fatigue MVC, and motor fatigue indices were detected with Mann-Whitney *U* tests.

Neuromuscular assessment data from two patients with MS were excluded from the analysis due to the patients' inability to match the force output to levels requested for cortical activation assessment. Distinct analyses of variance (ANOVAs) were run to test the effect of 'group' (HC, MS patients) and 'block' factors (25, 50, 75, 100% task failure) on changes in MVC (as a % of pre-fatigue values), pre-TMS EMG, peripheral, central, and supraspinal fatigue, MEP amplitude and latency, and CSP.

We compared the pre-TMS EEG activity between groups and conditions by means of a mixed ANOVA on the mean GMFP calculated on the 100 ms before the TMS pulse. To analyze LMFA and GMFP, we identified the time window of interests (TOI) from grand averages calculated across groups and conditions for each variable. TOI were selected around the main peaks identified by visual inspection on grand averages, with boundaries identified as throughs between peaks. We identified the following three TOI for LMFA: 15–25 ms, 25–45 ms and 45–60 ms post-TMS, and for GMFP: 15–23 ms, 24–34 ms and 35–60 ms

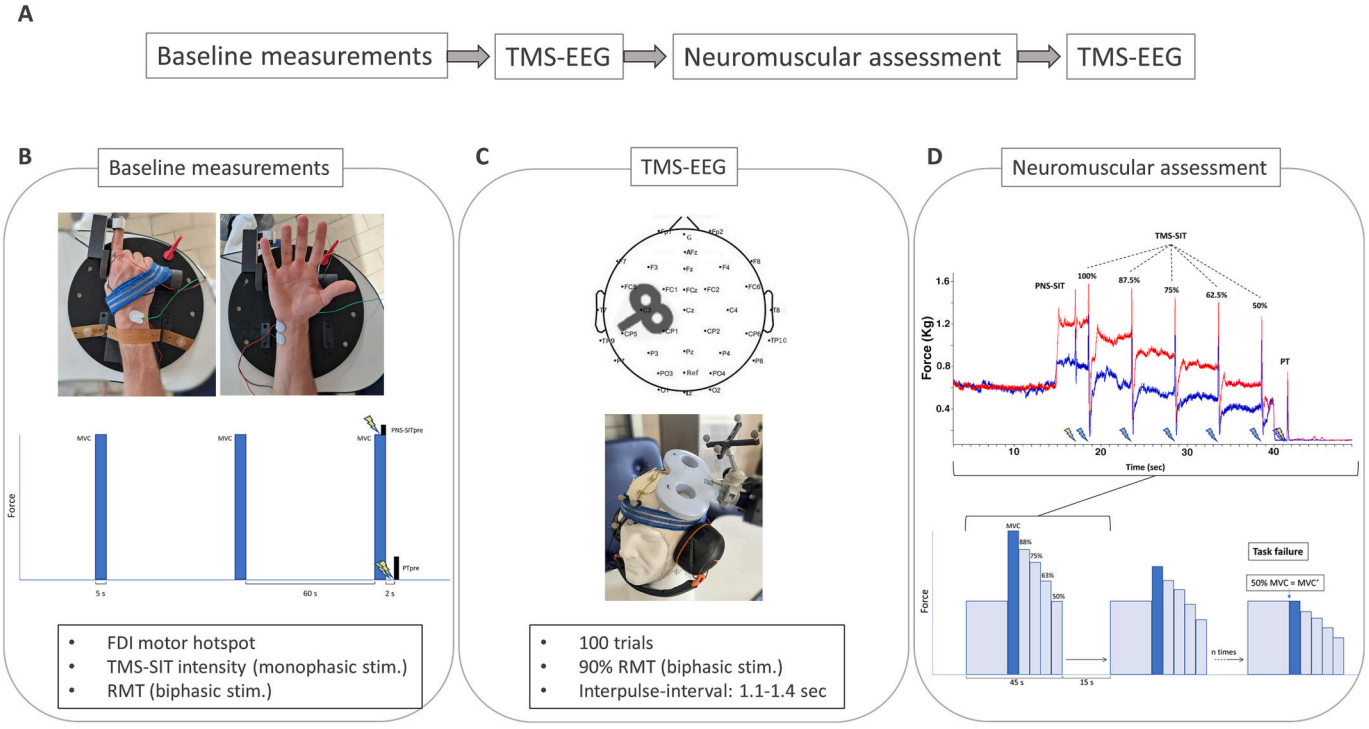

Fig. 1. Materials and methods.
(A) Experimental paradigm. (B) Experimental setup (top); schematic representation of the procedures for determining maximal voluntary contraction force (MVC), peripheral nerve stimulation-evoked superimposed twitch (PNS-SIT), and post-twitch (PT) (middle), as well as the first dorsal interosseous (FDI) hotspot by transcranial magnetic stimulation (TMS), TMS-evoked superimposed twitch (TMS-SIT), and rest motor threshold (RMT) (bottom). (C) Electrode placements of 32 TMS-compatible EEG channels, ground (G) and online reference (Ref) according to the international 10–20 system (top); TMS-EEG set-up (middle), and TMS-EEG session stimulation parameters (bottom). (D) Force recordings at different MVC levels during neuromuscular assessment blocks at task beginning (red) and close to task failure (blue) from one representative MS patient; lightning indicating the timing of PNS (yellow) and TMS (blue) pulses (top); schematic representation of the repetition of neuromuscular assessment blocks until task failure (bottom). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 3A). Peak identification was then performed automatically at a single subject level within each TOI, and repeated measure ANOVAs were run on peaks amplitude values (base-to-peak) with factors 'group', 'TOI' and 'condition' (pre- and post-fatigue). The sphericity was verified by Mauchly's tests, and the Greenhouse-Geisser correction was applied when necessary. Post-hoc analysis was conducted when appropriate by means of planned simple contrasts corrected for multiple comparisons by the false discovery rate when needed.

Statistical analysis for TEP source activation was carried out over current density time series values averaged across a region of interest (ROI) within the resting-state sensorimotor network of the stimulated hemisphere and across a TOI between 15 and 60 ms post-TMS (details are provided in the supplementary materials). We computed the source activation difference between pre- and post-fatigue TMS-EEG blocks (post-pre) for each participant. Finally, TEP source activation and pre- and post-fatigue differences between HCs and patients with MS were compared using an unpaired *t*-test permutation-based approach.(Pantazis et al., 2005)

We used Spearman's rank to investigate correlations between clinical fatigue scores, motor fatigue index, peripheral, central, and supraspinal fatigue at task failure, and TEP source activation differences between pre- and post-fatigue.

Unless otherwise specified, all values are expressed as mean ± standard deviation (SD).

2.9. Sample size calculation

No data is available on supraspinal fatigue in MS. A previous study compared central fatigue at task failure between MS and HCs and showed a large effect size.(Sheean et al., 1997) Since supraspinal fatigue is only a component of central fatigue and may be prone to more considerable variability due to its more significant dependency on complex cortical dynamics, we expected a medium effect size of 0.25. Since the primary hypothesis was tested by the block*group interaction of the 2 × 4 ANOVA, by setting an alpha of 0.05 and assuming an effect size of 0.25, there is a 90% probability chance of correctly rejecting the null hypothesis of no significant effect of the interaction on supraspinal fatigue with 15 patients in each group.

3. Results

3.1. Clinical, demographic, and stimulation variables

We found no significant differences in the proportion of males/females (Fisher's exact test $P = 0.053$) or in median age (34 vs. 35 years; $U = 113$, $P = 0.109$) between HCs and patients with MS. Furthermore, we found no significant differences in PNS intensity (median 38 vs. 35 mA, $P = 0.959$), TMS-SIT intensity (median 70 vs. 70 maximal stimulator output (MSO) %, $P = 0.646$), RMT (median 64 vs. 64 MSO%, $P = 0.610$), or pre-fatigue MVC (median 2.19 vs. 2.04 Kg, $P = 0.881$) values between HCs and patients with MS.

3.2. Fatigue measures

Median motor fatigue index scores (z-scores) were significantly higher in patients with MS (0.17) than in HCs (−0.6) ($P = 0.02$) (Fig. 2A, top). We found no significant differences in pre-fatigue MVC (median 2.19 vs. 2.04 Kg, $P = 0.881$) values between HCs and patients with MS (Fig. 2A, middle), and no significant 'group*block' interaction on MVC changes (%) ($P = 0.736$) (Fig. 2A, bottom). We found a significant 'group*block' interaction on central fatigue ($P = 0.021$) and supraspinal fatigue values ($P = 0.002$), but no significant interaction on peripheral

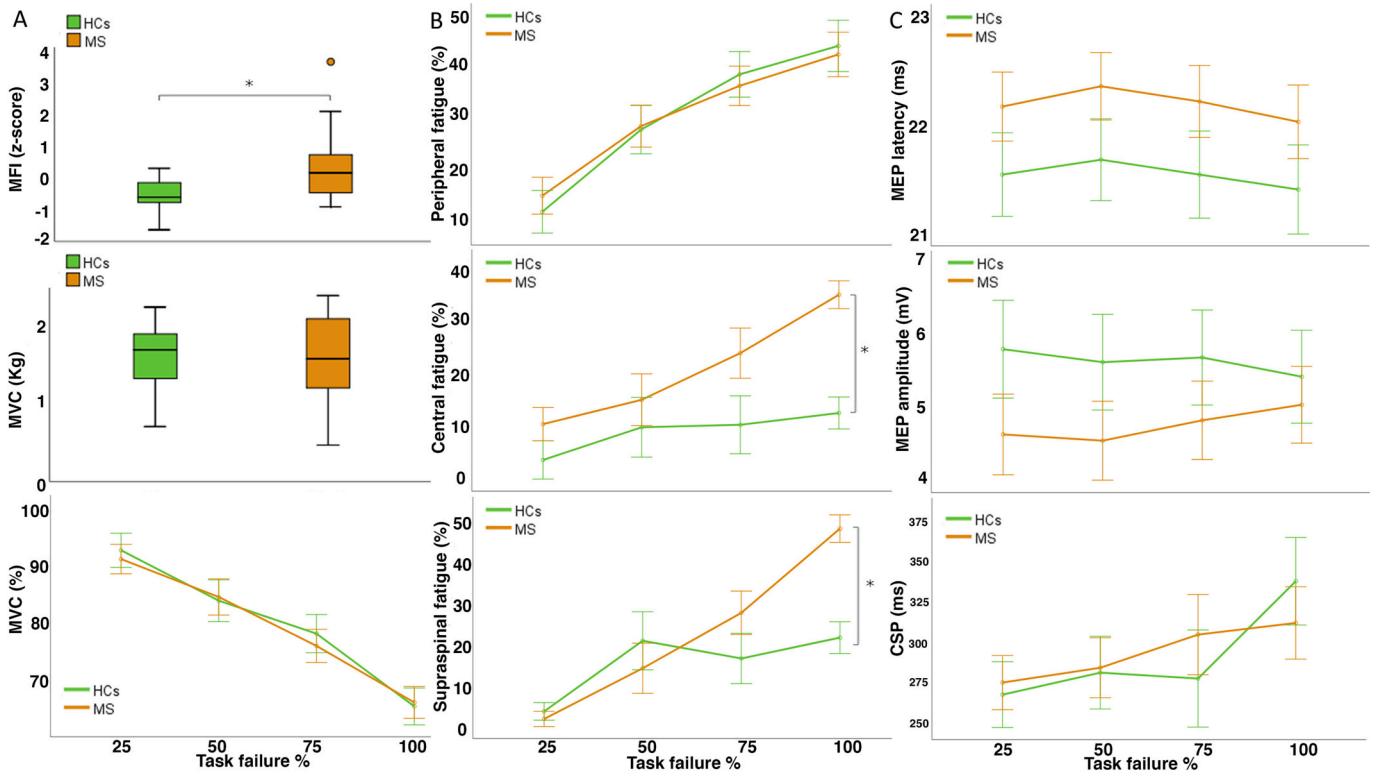

Fig. 2. Neuromuscular assessment results.
(A) Motor fatigue index (MFI) values (top) and maximal voluntary contraction (MVC) force values at baseline (i.e., pre-fatigue) (middle) and percentage changes per quarter of task duration before task failure (task failure) (bottom). (B) Percentage changes in peripheral fatigue (top), central fatigue (middle), and supraspinal fatigue (bottom) per quarter of task duration before (task failure). (C) Onset latency of motor evoked potentials (MEP) (top); MEP amplitude (middle), and cortical silent period (CSP) duration (bottom) during the task. Data reflect group averages. Error bars: standard error. *: significant differences between groups for $p < 0.05$.

fatigue ($P = 0.608$) (Table 2). In MS patients, there was a significant main effect of the 'block' factor on central ($F(3,30) = 22.36$, $P < 0.001$) and supraspinal fatigue ($F(3,30) = 32.15$, $P < 0.001$); in contrast, in HCs there was no significant effect of the 'block' factor on central ($F(3,42) = 1.76$, $P = 0.17$) or supraspinal fatigue ($F(3,42) = 2.06$, $P = 0.14$). Moreover, we found a significant main effect of the 'group' factor on central ($F(1,24) = 10.50$, $P = 0.003$) and supraspinal fatigue ($F(1,24) = 18.07$, $P < 0.001$) only at task failure and not before. Post-hoc comparison showed that at task failure MS patients had significantly higher central (36.64 ± 5.47 vs. 13.30 ± 4.68%, $P = 0.03$) and supraspinal fatigue (45.39 ± 4.37 vs. 20.96 ± 3.74%, $P < 0.001$) if compared to HCs (Fig. 2B).

3.3. TMS-EMG measures

We found no significant 'group*block' interaction on pre-TMS EMG ($P = 0.167$), MEP latency ($P = 0.978$), amplitude ($P = 0.201$), or CSP ($P = 0.331$). We identified a significant impact of the 'block' factor on CSP ($P = 0.019$), which was explained by a significantly longer CSP at task failure (309.78 ± 15.78 ms) compared to 25% task failure (271.41 ± 12.66 ms, $P = 0.001$) and 50% task failure (283.32 ± 13.89 ms, $P = 0.014$). We found no significant main effect of the 'group' factors on pre-TMS EMG ($P = 0.70$), MEP amplitude (HCs 6.01 ± 2.35 mV, MS 5.17 ± 2.36 mV), latency (HCs 21.80 ± 1.34 ms, MS 22.45 ± 1.35 ms), or CSP (HCs 290.18 ± 83.84 ms, MS 293.11 ± 84.24 ms) and no significant effect of 'block' on MEP amplitude and latency (all $P > 0.2$) (Fig. 2C) (Table 2).

3.4. TMS-EEG measures

We found no significant group*condition interaction ($P = 0.763$) or simple main effect for group ($P = 0.104$) on pre-TMS EEG as measured by the GMFP. We found no significant TOI*condition*group interaction ($P = 0.63$) or Condition*Group interaction ($P = 0.22$) effects on LMFA values. For GMFP analysis, we found no significant TOI*Condition*Group interaction ($P = 0.94$), but a significant Condition*Group interaction ($P = 0.03$). Post-hoc tests showed a trend for significant reduction in GMFP across all TOI in HCs at post-fatigue compared to pre-fatigue ($P = 0.09$) compared to a no significant increase in MS patients ($P = 0.115$) (Fig. 3). In both MS and HCs, pre-fatigue EEG source activation in the 60 ms after TMS predominantly projected around the stimulated central sulcus, with maximal values close to the stimulation site (corresponding to our ROI). TMS-evoked source activation also propagated over other areas of the sensorimotor network, including the ipsilateral frontal and posterior parietal cortex, and contralateral central sulcus. We found no significant differences in TMS-evoked source activation pre-fatigue between HCs and patients with MS. However, we noticed a significant activation difference within the ROI in pre- and post-fatigue in the time window between 44 and 52 ms explained by an increase in TMS-evoked source activation in MS patients at post-fatigue compared to pre-fatigue (2.7 ± 4.07) and to a significant reduction found in HCs (−0.22 ± 0.55) (Fig. 4).

3.5. Correlations

Correlations results are detailed in Table 3. One outlier for motor fatigue index values (MS patient) was removed from the analysis. FSIQ scores for the impact of fatigue on patients' lives positively correlated with motor fatigue index values. FSMC scores, MFIS scores, and FSIQ scores for fatigue-related symptom severity were not significantly correlated with any quantitative fatigue measure. Motor fatigue index values were positively correlated with supraspinal fatigue values and showed a trend for a negative correlation with peripheral fatigue values. Also, central fatigue was positively correlated with supraspinal fatigue,

Table 2
Main ANOVA results.

Factor	df[a]	F[b]	p	Factor	df	F	p
Fatigue measures							
Maximal Voluntary Contraction				**Peripheral Fatigue**			
Block	3,99	97.87	<0.001	Block	3,99	62.81	<0.001
Block*Group	3,99	0.42	0.736	Block*Group	3,99	0.55	0.608
Group	1,33	0.02	0.882	Group	1,33	<0.001	0.970
Central Fatigue				**Supraspinal Fatigue**			
Block	3,99	11.30	<0.001	Block	3,99	19.38	<0.001
Block*Group	3,99	3.38	0.021	Block*Group	3,99	6.08	0.002
Group	1,33	6.16	0.018	Group	1,33	2.74	0.107
TMS-EMG measures							
MEP[c]latency				**MEP amplitude**			
Block	3105	1.58	0.199	Block	3105	0.302	0.824
Block*Group	3105	0.07	0.978	Block*Group	3105	1.570	0.201
Group	1,35	1.73	0.198	Group	1,35	1.153	0.290
CSP[d]duration							
Block	3105	11.30	0.019				
Block*Group	3105	3.38	0.331				
Group	1,35	6.16	0.874				
TMS-EEG measures							
Local mean field amplitude (LMFA)				**Global mean field power (GMFP)**			
TOI	2,92	16.16	0.001	TOI	2,92	18.37	0.001
TOI*Group	2,92	0.37	0.69	TOI*Group	2,92	1.98	0.14
Condition	1,46	0.03	0.97	Condition	1,46	0.14	0.71
Condition*Group	1,46	1.55	0.22	Condition*Group	1,46	3.51	0.03
TOI*Condition	2,92	0.88	0.42	TOI*Condition	2,92	0.89	0.41
TOI*Condition*Group	2,92	0.43	0.63	TOI*Condition*Group	2,92	2.48	0.94
Group	1,46	0.18	0.67	Group	1,46	0.87	0.36

[a] Degrees of freedom.
[b] F-test.
[c] Motor evoked potentials.
[d] Cortical silent period.

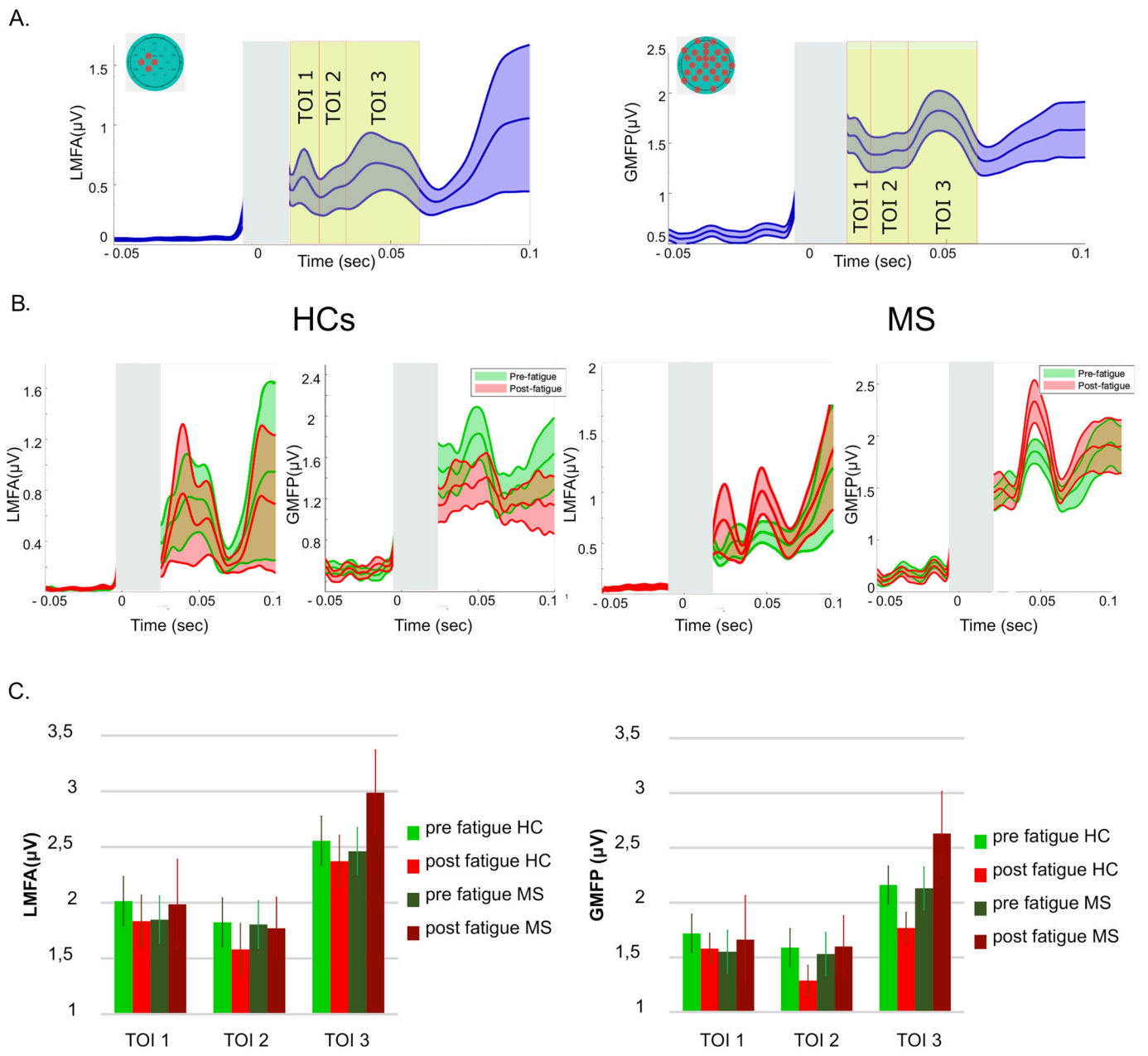

Fig. 3. TMS-EEG results – local mean field amplitude (LMFA) and global mean field power (GMFP).
(A). LMFA (left) and GMFP (right) grand averages across conditions and groups are depicted in blue (mean ± SEM). The three time-windows of interest (TOI) are highlighted in green. The electrodes used for each analysis are depicted in the top left corner of each plot. (B). LMFA and GMFP averages values (mean ± SEM) in pre- (green) and post-fatigue (red) conditions in healthy controls (HCs) and patients with multiple sclerosis (MS). (C). Histograms representing peak amplitude values (mean ± SEM) within each TOI. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

but was not significantly correlated with peripheral fatigue. Finally, the TMS-evoked source activation difference between pre- and post-fatigue (post-pre) was positively correlated with supraspinal fatigue but was not significantly correlated with peripheral or central fatigue values (Fig. 5).

4. Discussion

In the present study, we first confirmed that MS patients had more motor fatigue than HCs, as objectively quantified during a fatiguing task consisting of intermittent sustained contractions of the index finger. Neuromuscular assessment during fatiguing contractions showed that the increased motor fatigue found in patients was due to central and supraspinal fatigue components rather than peripheral mechanisms. The similar MEP amplitude, latency, and CSP recorded during the fatiguing contractions between patients and HCs suggests that in patients central motor fatigue was not due to either change in corticospinal excitability or transmission along the corticospinal tract. TMS-evoked superimposed twitches showed a suboptimal M1 output in MS patients compared to HCs. Patients and HC did not differ in fatigue-induced changes in local circuit excitability as measured by TEPs local mean field amplitude (LMFA). TEPs global mean field power (GMFP) indicated that fatigue in MS patients led to an increase in the spread of TMS-evoked activity from M1 to the rest of the cortex, in contrast to the decrease observed in HCs. We also found a post-fatigue increase in source-reconstructed TMS-evoked sensorimotor network activation following M1 stimulation in patients compared to the reduction observed in HCs, indicating that motor fatigue in MS is associated with an abnormal modulation of M1 connectivity within the sensorimotor network. Finally, in patients there

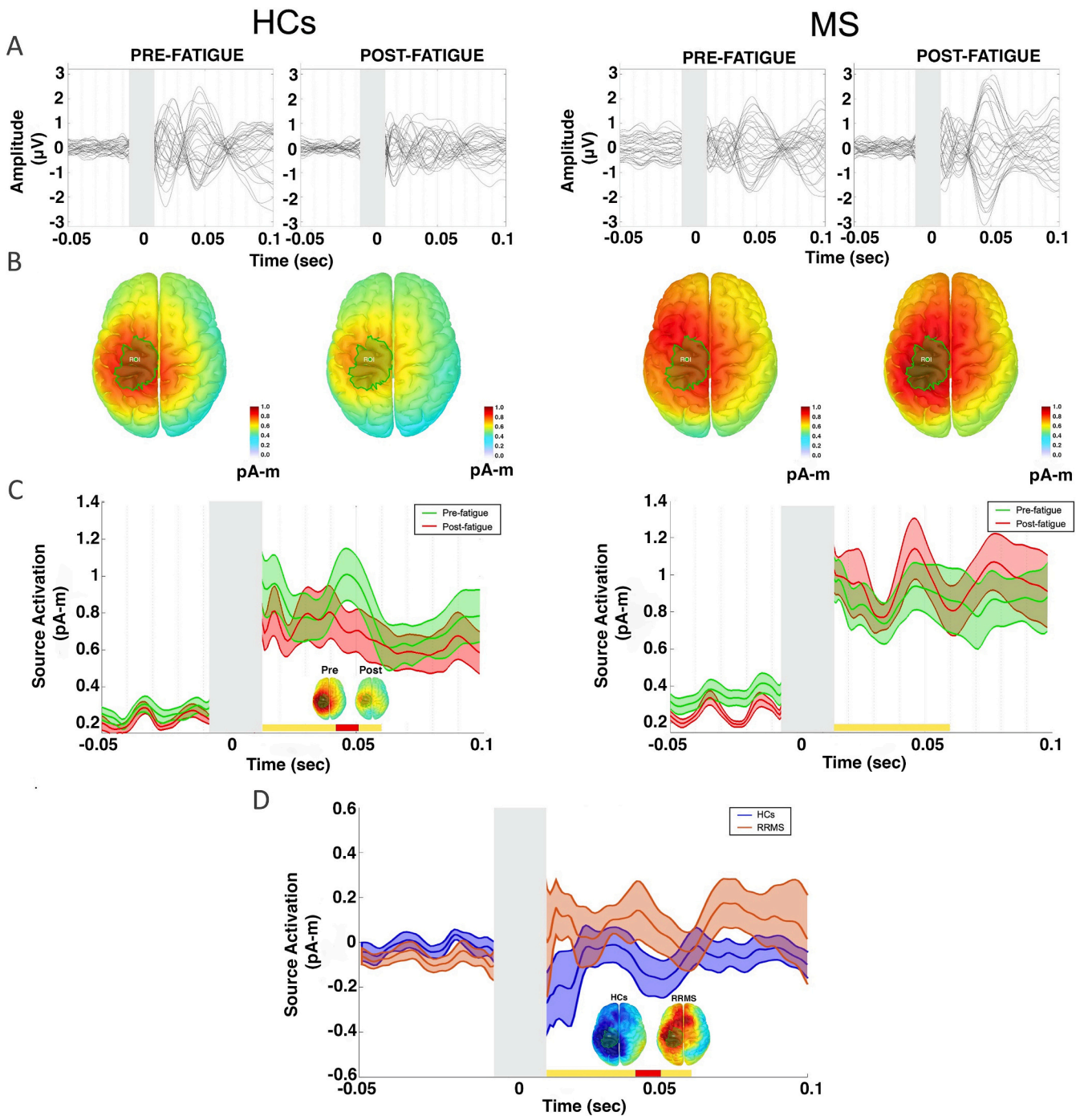

Fig. 4. TMS-EEG results – source reconstruction.
(A) Butterfly plots of grand average TEPs from left M1 stimulation re-referenced to average; grey bars: interpolated signal. (B) Source reconstruction of grand average TMS-evoked cortical activation in our time window of interest (TOI) between 15 and 60 ms; green-shaded correspond to our region of interest (ROI). (C) Time series of grand average TMS-evoked cortical activation within the ROI in the sensorimotor network; the yellow bar represents the TOI; (left) the time interval showing significant differences between pre- and post-fatigue is highlighted in red and brain topographies within this time interval are provided. (D) Time series of grand average TMS-evoked cortical activation differences between pre- and post-fatigue (post – pre), within the ROI. The yellow bar represents the TOI; the time interval showing significant differences between HCs and MS patients is highlighted in red and brain topographies within this time interval are provided. Data reflect group averages. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was an association between subjectively reported impact of fatigue on daily activities and objectively quantified motor fatigue, and participants displaying higher supraspinal fatigue also showed higher motor fatigue and higher post-fatigue increase in TMS-evoked activation within the sensorimotor network. Overall, the results of the present study suggest that motor fatigue in MS reflects a reduced M1 output (supraspinal fatigue) as well as an abnormal task-related modulation of sensorimotor network dynamics.

We took several methodological precautions to control for possible confounders. Since MS patients and HCs showed similar age, gender distribution, and stimulation parameters, we may tentatively exclude the possibility that these factors could have affected the interpretation of our results. To limit the heterogeneity of disease-modifying treatment and disease activity in the patient sample, we enrolled only patients

Table 3
Spearman correlation coefficients (p).

	MFI	PF	CF	SF	TMS-SA$_{diff}$
MFIS	0.398 (0.091)	−0.147 (0.537)	−0.137 (0.565)	0.009 (0.970)	0.325 (0.188)
FSMC	0.293 (0.223)	−0.003 (0.990)	−0.270 (0.250)	−0.064 (0.789)	0.299 (0.228)
FSIQ[a]	0.417 (0.076)	−0.268 (0.254)	0.189 (0.424)	0.282 (0.229)	0.074 (0.769)
FSIQ[b]	**0.541 (0.017)**	−0.356 (0.123)	−0.061 (0.798)	−0.088 (0.712)	0.151 (0.549)
MFI	–	−0.301 (0.084)	0.252 (0.151)	**0.410 (0.016)**	0.140 (0.420)
PF	–	–	−0.146 (0.402)	−0.052 (0.768)	0.172 (0.347)
CF	–	–	–	**0.618 (<0.001)**	0.289 (0.108)
SF	–	–	–	–	**0.386 (0.029)**

Modified Fatigue Impact Scale (MFIS); Fatigue Scale for Motor and Cognitive Functions (FSMC); Fatigue Symptoms[a] and Impacts[b] Questionnaire – Relapsing Multiple Sclerosis (FSIQ); Motor fatigue index (MFI); Peripheral fatigue (PF); Central fatigue (CF); Supraspinal fatigue (SF); TMS-evoked source activation difference (post-pre) (TMS-SA$_{diff}$).

chronically treated with natalizumab. We excluded patients with upper motoneuron signs in the right arm to exclude possible confounding effects due to corticospinal tract alterations. In addition, we excluded patients with depression to limit confounding effects on volitional effort during the task. In order to compare the contribution of peripheral, central, and supraspinal fatigue between groups in matched functional conditions, all subjects were tested until task failure, and we adjusted the motor fatigue index for MVC values.(Steens et al., 2012b) The instruction to restore as quickly as possible the required force level during the task further limited confounders due to differences in behavioral output. Since patients and HCs had similar baseline MVC values and linear decline in MVC values during the fatiguing task, we may tentatively exclude confounders due to motivation differences between groups during task execution. The findings that pre-TMS EMG and EEG values were similar between patients and HCs exclude that possible differences in background EMG and EEG activity may confound our results. Finally, since TEPs were elicited using below-motor-threshold stimulation intensities, we may exclude possible confounding due to reafferent activity associated with MEP-related muscle twitch.

The first finding of the present study is that patients and HCs displayed similar values in peripheral fatigue at task failure, suggesting that neither defective neuromuscular transmission(Patten et al., 1972) nor muscular activity(Kent-Braun et al., 1994; Lenman et al., 1989; Miller et al., 1990; Vaz Fragoso et al., 1995) explains motor fatigue in MS patients. The higher central component of motor fatigue we found in patients in comparison with HCs suggests an impaired descending drive possibly due to a defective corticospinal transmission or suboptimal M1 output.(Gandevia, 2001; Peters and Fuglevand, 1999) Since we found no differences in central activation, as measured by the superimposed twitch between patients and HCs at baseline, we may tentatively exclude that a possible corticospinal abnormality explains the increased central motor fatigue in patients. The fact that there were no differences in MEP amplitude or latency between patients and HCs at baseline or during the task suggests that central motor fatigue does not depend on exercise-induced changes in spinal motoneuron excitability, corticospinal tract conduction, or the excitability of the circuit projecting on pyramidal tract neurons at M1 level. Furthermore, there was a similar exercise-induced increase in CSP duration in patients and HCs, implying that changes in GABA-B-mediated intracortical inhibition of pyramidal tract neurons do not explain increased central fatigue in patients.(Kang et al., 1994; Mills and Thomson, 1995; Taylor et al., 1999) Our results do not

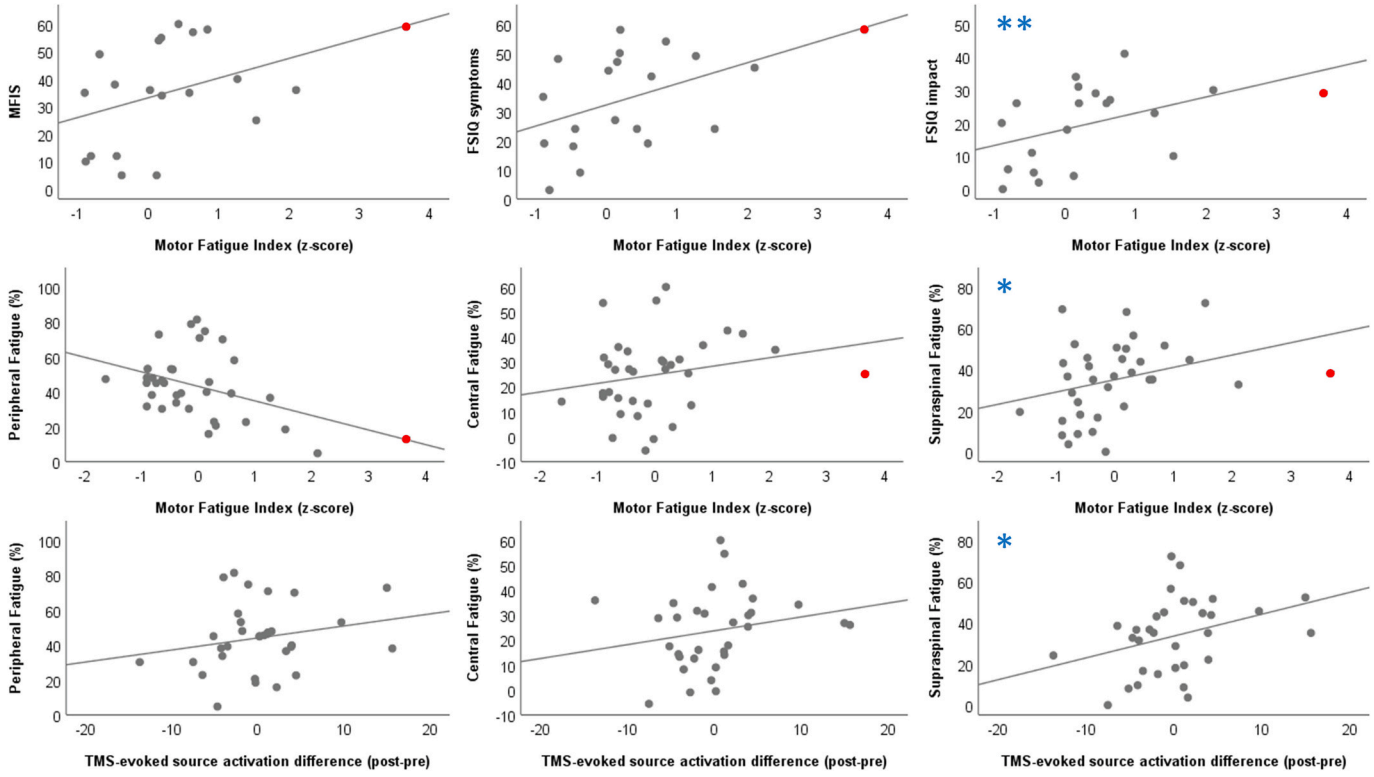

Fig. 5. Correlation scatterplot.
MFIS: Modified Fatigue Impact Scale. FSIQ: Fatigue Symptoms and Impacts Questionnaire. Red point: removed outlier. *: Spearman's correlation coefficient significant for $p < 0.05$; **: $p < 0.01$. Line: linear trend. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

back up the results of a previous study reporting more significant increases in MEP amplitude and CSP duration in patients with MS compared to HCs.(Thickbroom et al., 2006) Differences between studies may be due to different clinical features of patients. Contrary to the present study, Thickbroom and colleagues(Thickbroom et al., 2006) reported on patients who had decreased baseline MEP amplitude and MVC, providing ground for impaired corticospinal integrity. Motor fatigue in MS has been previously associated with reduced intracortical inhibition, as measured by MEP inhibition in response to the paired-pulse stimulation paradigm.(Mainero et al., 2004; Liepert et al., 2005) However, in their study the authors tested the patients at rest and not during a fatiguing contraction, and thus, it is unclear whether reduced intracortical inhibition may also contribute to motor fatigue in MS. Despite normal corticospinal transmission, our findings of higher supraspinal fatigue compared to HCs supports the hypothesis that central motor fatigue in patients reflects defective M1 output due to a limiting process upstream for trans-synaptic activation of pyramidal tract neurons. The supraspinal fatigue results of the present study are in line with a recent study from Brotherton and colleagues,(Brotherton et al., 2022) supporting the role of a reduced M1 output in motor fatigue in MS.

Differently from the TMS-evoked superimposed twitch recorded during the task reflecting changes in M1 output, the TMS-EEG measures we collected (TEPs local mean field amplitude (LMFA), global mean field power (GMFP), and TMS-evoked source activity) provide insight into how the reduction in M1 output underlying motor fatigue may reflect changes in M1 excitability and connectivity in MS. TEPs reflect both summations of excitatory and inhibitory post-synaptic potentials and action potentials propagation via anatomical connections specific for the stimulated area, preferentially within the functional network to which the area belongs.(Bortoletto et al., 2015; Esposito et al., 2022; Leodori et al., 2022a; Leodori et al., 2020; Ozdemir et al., 2020; Tremblay et al., 2019; Momi et al., 2021; Esposito et al., 2020) Therefore, TEPs allow obtaining indices of excitability and effective connectivity of the stimulated area. Among TMS-EEG indices, the local mean field amplitude is thought to reflect signal propagation within local circuits and has been associated with local excitability changes.(Tremblay et al., 2019; Leodori et al., 2019) The global mean field power reflects signal propagation to remote areas and therefore provide an index of global cortical excitability as a function of effective connectivity of the stimulated area with the rest of the brain.(Tremblay et al., 2019; Esser et al., 2006) Finally, the TMS-evoked source activity measured within a functional network is considered an index of effective connectivity of the stimulated area with other nodes within the functional network.(Bortoletto et al., 2015; Esposito et al., 2022; Ozdemir et al., 2020; Momi et al., 2021; Esposito et al., 2020) Since we found that TEPs at baseline were similar between patients and HCs, we can exclude that MS-related brain structural abnormalities explain differences in task-related TEPs modulation.(McDonald and Sears, 1970; Rasminsky and Sears, 1972; van der Werf et al., 1998) Local mean field amplitude and global mean field power results suggest that MS-related fatigue is associated with an abnormal modulation of M1 effective connectivity rather than local excitability. The spatiotemporal profile of source-reconstructed TEPs in the present study supports the hypothesis that cortical activation evoked by TMS over M1 stimulation preferentially propagates within the ipsilateral and contralateral area belonging to the sensorimotor network. The correlation we found between supraspinal fatigue and source-reconstructed TEPs supports the hypothesis that M1 output reduction during contraction is related to an abnormal task-induced modulation of M1 connectivity within the sensorimotor network that can have different pathophysiological meanings. Post-fatigue TEP facilitation in MS patients may reflect an increase in M1 connectivity within the sensorimotor network during the motor task to compensate for MS-related structural abnormalities.(Steens et al., 2012a; Reddy et al., 2000; Rocca et al., 2005) However, if post-fatigue TEP facilitation reflected a compensatory increase in M1 connectivity to maintain M1 output, it would have been inversely correlated with supraspinal fatigue values, while we observed a direct correlation. Furthermore, TEP facilitation was found during the post-exercise rest phase, whereas an increase in connectivity aimed to maintain M1 output would have been expected during the task. Alternatively, TEP facilitation in patients may reflect an impairment of post-exercise recovery mechanisms aimed at inhibiting the system at rest.(Thickbroom et al., 2006) In this vein, post-exercise TEP facilitation in patients may reflect persistence, at rest, of task-related increase in M1 connectivity. This hypothesis is in line with the increase in resting sensorimotor network activity previously reported in MS.(Thickbroom et al., 2006; Reddy et al., 2000; Rocca et al., 2005) The increased overall cortical activation following a fatiguing task, as measured by the global mean field power and source-reconstructed TEPs, may be one of the mechanism underlying the disproportionate effort perception in attempted activities often reported by MS patients.(Kluger et al., 2013) Furthermore, the persistence of task-related activation in MS patients following the task may be a possible mechanism of task-switching difficulties previously described in these patients.(Migliore et al., 2018) Several factors may contribute in MS to this abnormal modulation of brain network activity in response to a sustained activation, including demyelination,(Freal et al., 1984) neuronal damage,(Induruwa et al., 2012) and neuroinflammation, (Heesen et al., 2006) as well as secondary factors such as mood and sleep disorders, iatrogenesis, and physical inactivity.(Chalah et al., 2015; Krupp et al., 2010; Newland et al., 2016) Previously described abnormalities in cortical plasticity in MS patients may also contribute to abnormal reorganization in M1 connectivity in response to a motor task. (Baione et al., 2020; Conte et al., 2016) Our TMS-EEG results are in line with fMRI studies in patients with MS showing an association between fatigue and increased task-induced brain activation,(Filippi et al., 2002) but not with others reporting lower sensorimotor cortical activation. (Steens et al., 2012a) Notwithstanding this, compared to fMRI measures, TMS-EEG correlates of sensorimotor network activity have several advantages. They are free from performance-related confounding since TEPs are recorded at rest, are sensitive to fast neural dynamics, and are specifically related to the functional state of stimulated M1.

Finally, we found a significant correlation only between FSIQ scores for the impact of fatigue on daily activity and motor fatigue index values. The FSIQ score relies on subjective patients reports, whereas the motor fatigue index provides objective measures of motor fatigue. Although the correlation we found between FSIQ scores and motor fatigue index values suggests the validity of our quantitative measures in explaining the mechanisms underlying subjective fatigue, it also suggests that self-reported fatigue and objective measures of motor fatigue may provide complementary information on MS-related fatigue. Our results highlight the importance of considering multiple methods when studying fatigue in MS patients.

We acknowledge several limitations. It is relevant to note that the absence of FSMC motor sub scores in this study limited our ability to investigate the correlation between experimentally induced motor fatigue and one of the clinical measures of motor fatigue in MS patients. Further research with larger and other samples is needed to establish the validity of the findings and their generalizability to patients with MS. Since MEP mainly reflects trans-synaptic activation of the fast-conducting pyramidal fibers mainly involved in fine and fast movements rather than in the sustained tonic contractions used in this study, (Evarts, 1968; Hepp-Reymond et al., 1974; Hepp-Reymond and Wiesendanger, 1972; Hess et al., 1987; Lawrence and Kuypers, 1968; Lemon et al., 1986) our TMS-EMG measures may have underestimated the possible contribution of slow-conducting pyramidal fiber activity changes to motor fatigue. Since TMS-EEG recording requires several minutes, we collected TEPs only before and after, and not during, the

fatiguing task. Therefore, we cannot exclude changes in TEP amplitude during the task and cannot determine the modulation onset we recorded post-fatigue. Similarly, since motor cortical excitability changes rapidly after fatigue,(Samii et al., 1996) the TEP testing time might have limited our ability to identify other differences between the two groups. However, previous studies have shown that corticospinal excitability is markedly depressed for half an hour after fatiguing exercise when tested at rest.(Brasil-Neto et al., 1994)

5. Conclusions

In conclusion, our study shows that motor fatigue in patients with MS is due to central mechanisms. Using a neuromuscular assessment and recording the muscle responses evoked by TMS of M1, we have provided novel evidence suggesting that motor fatigue in these patients is due to a suboptimal M1 output. Furthermore, by adopting a TMS-EEG approach, we have provided evidence suggesting that motor fatigue in MS patients is associated with impaired recovery mechanisms within the sensorimotor network related to abnormal modulation of M1 excitability and connectivity. Our results provide significant advancements toward understanding the pathophysiology of motor fatigue in patients with MS, which may be used to develop new treatment strategies for this common and yet poorly treated symptom.

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CRediT authorship contribution statement

Giorgio Leodori: Conceptualization, Methodology, Investigation, Writing – original draft, Formal analysis. **Marco Mancuso:** Visualization, Investigation, Formal analysis, Writing – original draft. **Davide Maccarrone:** Visualization, Data curation, Investigation, Software. **Matteo Tartaglia:** Data curation, Investigation, Data curation. **Antonio Ianniello:** Investigation, Resources, Data curation. **Francesco Certo:** Investigation, Formal analysis. **Viola Baione:** Investigation, Resources, Data curation. **Gina Ferrazzano:** Resources, Data curation. **Leonardo Malimpensa:** Resources, Data curation. **Daniele Belvisi:** Supervision, Conceptualization, Writing – review & editing. **Carlo Pozzilli:** Supervision, Conceptualization, Writing – review & editing, Project administration, Funding acquisition. **Alfredo Berardelli:** Supervision, Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Antonella Conte:** Supervision, Conceptualization, Methodology, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors report no competing interests.

Data availability

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

Appendix A. Supplementary data

Supplementary material is available at *Neurobiology of Disease* online. Supplementary data to this article can be found online at [https://doi.org/10.1016/j.nbd.2023.106073].

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