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Insight into motor fatigue mechanisms in natalizumab treated multiple sclerosis patients with wearing off

Giorgio Leodori[1,2]✉, Marco Mancuso[2], Davide Maccarrone[2], Matteo Tartaglia[1,2], Antonio Ianniello[1,2], Francesco Certo[2], Gina Ferrazzano[2], Leonardo Malimpensa[1,2], Daniele Belvisi[1,2], Carlo Pozzilli[2], Alfredo Berardelli[1,2] & Antonella Conte[1,2]

Motor fatigue in Multiple Sclerosis (MS) is due to reduced motor cortex (M1) output and altered sensorimotor network (SMN) modulation. Natalizumab, a disease-modifying therapy, reduces neuroinflammation and improves fatigue. However, some patients treated with natalizumab experience fatigue recurrence ('wearing-off') before subsequent infusions. Wearing-off provides a valuable window into MS-related motor fatigue mechanisms in a controlled, clinically stable, setting. This study investigates whether wearing-off is associated with worsening motor fatigue and its neurophysiological mechanisms and assesses natalizumab's effect on MS-related fatigue. Forty-five relapsing–remitting MS patients with wearing-off symptoms were evaluated pre- and post-natalizumab infusion. Assessments included evaluating disability levels, depressive symptoms, and the impact of fatigue symptoms on cognitive, physical, and psychosocial functioning. The motor fatigue index was computed through the number of blocks completed during a fatiguing task and peripheral, central, and supraspinal fatigue (M1 output) were evaluated by measuring the superimposed twitches evoked by peripheral nerve and transcranial magnetic stimulation of M1. Transcranial magnetic stimulation-electroencephalography assessed M1 effective connectivity by measuring TMS-evoked potentials (TEPs) within the SMN before- and after the task. We found that wearing-off was associated with increased motor fatigue index, increased central and supraspinal fatigue, and diminished task-related modulation of TEPs compared to post-natalizumab infusion. Wearing-off was also associated with worsened fatigue impact and depression symptom scores. We conclude that the wearing-off phenomenon is associated with worsening motor fatigue due to altered M1 output and modulation of the SMN. Motor fatigue in MS may reflect reversible, inflammation-related changes in the SMN that natalizumab can modulate. Our findings apply primarily to MS patients receiving natalizumab, emphasizing the need for further research on other treatments with wearing-off.

Keywords Motor fatigue, Natalizumab, Wearing-off, Neurophysiology, TMS-EEG, Sensorimotor network

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of the central nervous system (CNS) and is one of the leading causes of disability in young adults[1]. Despite the availability of numerous disease-modifying therapies (DMTs) to control acute central nervous system inflammation, some hidden symptoms remain challenging to treat, significantly impacting patients' quality of life[2]. Fatigue is one of the most common and debilitating symptoms of MS, defined as either a subjective decrease in energy levels or a disproportionate perception of effort during attempted or general activities[3]. Fatigue includes a motor component, defined as a progressive decline in muscle strength during ongoing or repetitive contractions[4–6]. Transcranial Magnetic Stimulation (TMS) can assess the output from the motor cortex (M1) by measuring TMS-evoked extra forces during fatiguing tasks[6]. Combining TMS with electroencephalography (TMS-EEG) enhances this assessment by enabling the measurement of TMS-evoked potentials (TEPs), which reflect M1 excitability and effective connectivity[7,8]. Our previous study[5] with neuromuscular assessments and TMS-EEG[8] suggested that motor

[1]IRCCS Neuromed, 86077 Pozzilli, IS, Italy. [2]Department of Human Neurosciences, Sapienza University of Rome, Viale Dell'Università, 30, 00185 Rome, Italy. ✉email: giorgio.leodori@uniroma1.it

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fatigue in MS is associated with a decreased output from M1 due to an abnormal task-related modulation of M1 effective connectivity within the sensorimotor network (SMN).

Clinical studies indicate that natalizumab treatment, a monoclonal antibody targeting the α4-integrin molecule preventing MS relapses, mitigates fatigue severity[9,10]. However, some patients report recurring fatigue symptoms, a clinical phenomenon known as 'wearing-off' that typically starts about 21 days after natalizumab infusion and generally improves approximately one day following the subsequent infusion[11]. Reduced natalizumab receptor occupancy on CD8 + and CD4 + effector memory T-cells at the end of the dosing interval may enable these cells to enter the central nervous system, leading to wearing-off symptoms[12]. T-cells may contribute to wearing-off by producing proinflammatory cytokines and exhibiting cytotoxic effects[13,14]. If motor fatigue and its neurophysiological correlates increase during the wearing-off period, this would suggest that neuroinflammatory mechanisms may contribute to motor fatigue in MS. Studying MS-related fatigue through the time window of the wearing-off phenomenon allows us to explore these dynamics in stable patients, thus avoiding the confounding factors of acute inflammatory activity and significant changes following treatment initiation. The wearing-off, therefore, presents a unique window to study the mechanisms underlying motor fatigue in MS. Since natalizumab-naive patients experience an improvement in fatigue symptoms after starting the treatment[10]; then, we expect the wearing-off fatigue to reflect the re-emergence of the same mechanisms underlying MS-related fatigue. Investigating the neurophysiological correlates of fatigue will also clarify how natalizumab modulates these processes.

Our study aims to clarify whether wearing-off fatigue in natalizumab-treated MS patients is associated with worsening in objectively assessed motor fatigue and its neurophysiological correlates. If wearing-off shares similar mechanisms to those underlying motor fatigue in MS, then a worsening of neurophysiological correlates associated with MS-related motor fatigue should be present during the wearing-off phase compared to post-natalizumab treatment.

To these aims, we compared motor fatigue index, M1 output, and fatigue-related changes in M1 effective connectivity in clinically stable patients with relapsing–remitting multiple sclerosis (RRMS) during the wearing-off and two weeks after natalizumab infusion.

Methods

Participants

We recruited 45 patients (average age 37.1 ± 9.1 years, 32 females) diagnosed with relapsing–remitting MS (RRMS) based on the revised McDonald criteria who presented wearing-off related fatigue[15]. To assess wearing-off-related fatigue, participants were asked whether they felt worse toward the end of the natalizumab dosing cycle compared to earlier in the cycle[11]. Participants provided written informed consent to participate in the study. All research procedures were approved by the institutional review board at Sapienza University of Rome (protocol 0768/2020) and were conducted following the Declaration of Helsinki.

Inclusion criteria required patients to report wearing-off fatigue symptoms the week before the Natalizumab scheduled dose, be between 18 and 65 years old, and have an Expanded Disability Status Scale (EDSS) score of less than 6.5. Participants with an EDSS > 6.5 were excluded due to the nature of the fatigue-inducing task, which requires maintaining high effort and concentration. Patients were required to have received five or more prior infusions of Natalizumab and had to be clinically and radiologically stable for at least one year. Also, only right-handed patients were selected to reduce variability in the neurophysiological measures due to brain lateralization patterns. Exclusion criteria were the presence of psychiatric disorders, including depressive symptoms with a Beck Depression Inventory-II (BDI-II) score > 19[16] to minimize the potential influence on task effort, clinical signs of upper right arm weakness, superficial or deep sensory loss, or upper limb spasticity and other neurological conditions to prevent confounding effects due to corticospinal and sensory pathway alterations. We did not include participants with contraindications for transcranial magnetic stimulation (TMS) or patients who had introduced or modified any medications, including those for mood, fatigue, or cognition, within the study period or in the preceding month.

Patients were studied within one week before (T0), i.e., the wearing-off phase, and two weeks after natalizumab infusion (T1), with the order of the two sessions randomized. Participants' demographics were obtained through direct patient interviews during the first visit. Clinical assessment at both time points included the 21-item Modified Fatigue Impact Scale (MFIS, range 0–84)[17], the EDSS, and the BDI-II. A certified neurologist assessed the EDSS through a neurological examination and clinical history review. The neurophysiological assessment was conducted at the same hour of the day (± 2 h) to account for diurnal variations in force and corticospinal excitability[18] (Fig. 1A).

Neurophysiological evaluation

The neurophysiological evaluation encompassed a neuromuscular assessment during a fatigue-inducing task and two blocks of TMS-EEG performed before and after the fatigue task. We employed the methodologies outlined in a recent publication, which will be detailed in the next paragraphs[7]. The neuromuscular assessment was carried out in order to quantify motor fatigue and distinguish between its peripheral, central (i.e., mechanisms both distal and proximal to the neuromuscular junction), and supraspinal (i.e., mechanisms related to changes in M1 output) components[6,19,20]. The TMS-EEG blocks were used to collect TMS-evoked potentials (TEPs) before and after the fatiguing task to assess M1 effective connectivity within the SMN.

Stimulation

TMS was delivered over the left M1 with a figure of eight coils on the hotspot of the first dorsal interosseous muscle (FDI) defined as detailed in previous research[21]. During the neuromuscular assessment, TMS was delivered

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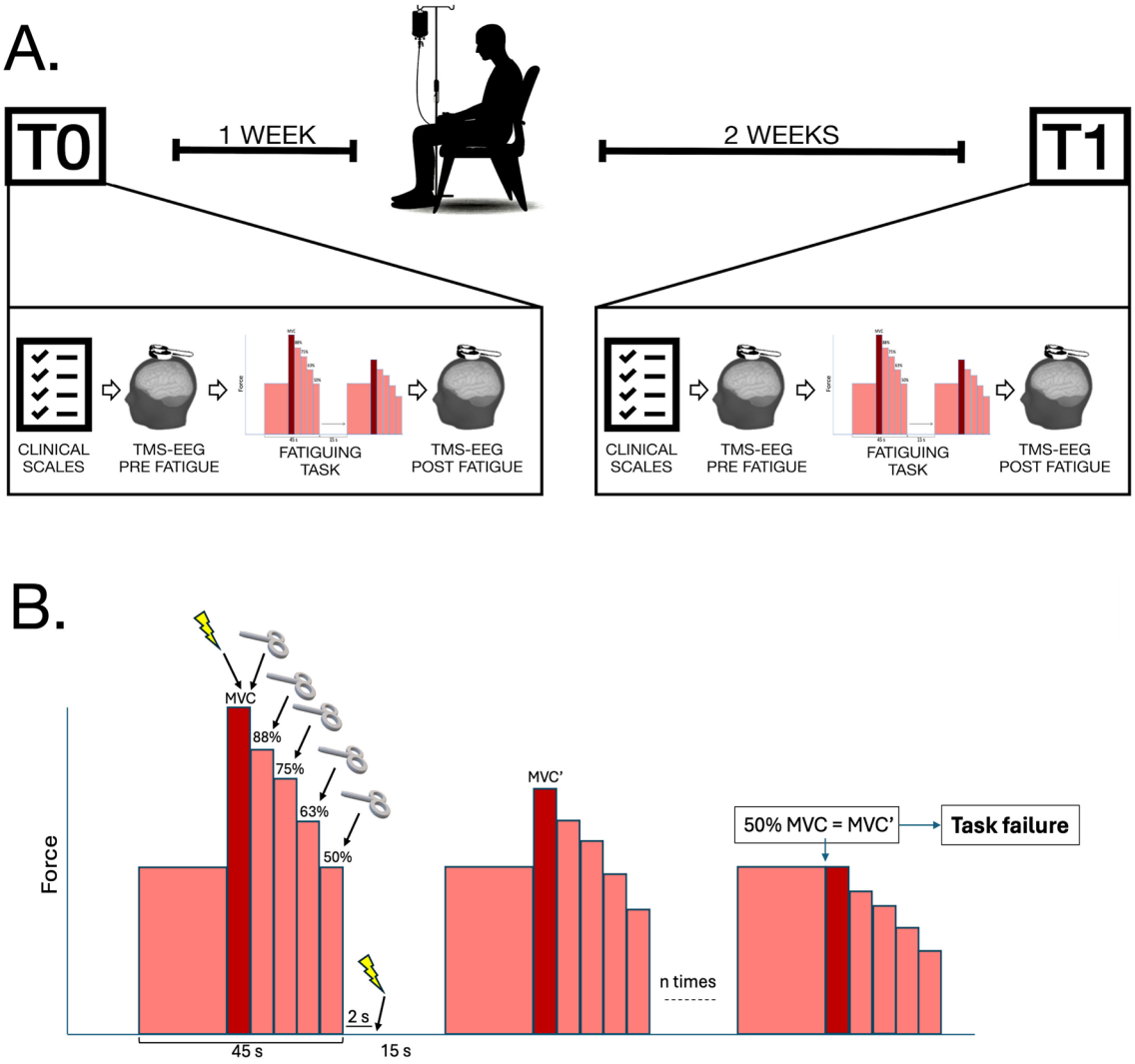

Figure 1. Study Protocol Overview. **A** Study Protocol Schematic: Participants were evaluated one week prior (T0) to and two weeks following (T1) natalizumab infusion, with the order of assessments randomized. Each session included clinical scale evaluations, a pre-fatigue TMS-EEG session, neuromuscular assessments during a fatiguing task, and a post-fatigue TMS-EEG session. **B** Fatiguing Protocol Schematic: The protocol involved consecutive blocks, each consisting of 45 s of continuous first dorsal interosseus (FDI) muscle contraction at varying force levels—100%, 88%, 75%, 63%, and 50% of maximal voluntary contraction (MVC), followed by 15 s of rest. Peripheral nerve stimulation (PNS) to the ulnar nerve (yellow bolts signs) was applied at 100% MVC and at rest, while TMS pulses (8-shape coil signs) were delivered at each force level. Task failure was defined as when the MVC for a block (MVC') fell to 50% of the baseline MVC. Abbreviations: MVC: maximal voluntary contraction, TMS-EEG: transcranial magnetic stimulation and electroencephalography.

using a monophasic stimulator (Magstim 200-2) to elicit TMS-evoked superimposed twitches (SIT). During the TMS-EEG session, TMS was delivered using a biphasic stimulator (Magstim, SuperRapid-2) to elicit TEPs. During the neuromuscular assessment, peripheral nerve stimulation (PNS) was delivered over the right ulnar nerve at the wrist using a constant-current stimulator (Digitimer DS7AH) to elicit a PNS-induced SIT and post-twitches (PT). The PNS was applied in pairs of stimuli with a 10-ms gap, where each stimulus had a duration of 200 microseconds (See Experimental procedures for further details).

Recording

A custom force transducer (EMS, Italy) measured the abduction force of the right index finger while the hand held a horizontal handle. Electromyography (EMG) was sourced from the right FDI muscle. EEG data was collected from 32 passive electrodes fitted on a cap (BrainCap, EASYCAP) adhering to the 10–20 system. The EEG signals underwent filtering between Dc-5 kHz and were sampled at a rate of 5 kHz with a TMS-compatible EEG amplifier (NeurOne, Bittium).

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Experimental procedures

Participants sat with their right forearm resting on a table, the right hand secured to force the transducer. The right index finger's maximal voluntary contraction force (MVC) was defined as the mean of three 3-s abduction trials. Using the biphasic stimulator, the resting motor threshold (RMT) for TMS-EEG was determined as the minimum stimulation intensity required to produce a motor-evoked potential (MEP) of at least 50 μV in the FDI in 5 out of 10 consecutive trials, while the participant was at rest [21,22]. By incrementing the maximal stimulator output percentage (MSO %) of the monophasic stimulator in 5% steps, we determined the TMS intensity for neuromuscular examination. This was based on the minimum MSO capable of inducing a superimposed twitch (SIT) during a 50% MVC contraction. The PNS intensity was set to 120% of the minimal intensity required to induce a post-twitch (PT) at rest.

The neuromuscular assessment consisted of performing blocks of 40 s of index finger abduction at 50% (15 s), 100% (5 s), 75% (5 s), 62.5% (5 s), and 50% (5 s) of the MVC recorded in the previous block (baseline MVC for the first block), followed by 10 s of rest (Fig. 1B). During each block, PNS was applied during maximum MVC to elicit a PNS-induced SIT and again post-contraction, after a 2-s rest, to produce a PT. Also, a TMS pulse was applied during each force step to induce a TMS-evoked SIT. The blocks continued until participants failed to maintain an MVC of at least half their initial values for over two seconds.

TMS-EEG sessions at rest were performed both before and immediately after the neuromuscular evaluation. This involved 100 trials of biphasic TMS at 90% RMT, interspaced by intervals of 1250 ± 10% ms, concurrently with continuous EEG monitoring. Participants wore noise-reducing earmuffs and earphones playing a sound specifically designed to mask the TMS click. A thin foam layer was placed beneath the coil to diminish auditory and tactile co-stimulation[23].

Data analysis

The motor fatigue index was determined as the inverse of the product of completed block numbers and the pre-fatigue MVC values. Motor fatigue index values were z-score normalized across participants. We measured the MVC for each block (MVC') as the average force exerted during the 100 ms period immediately preceding the PNS. We measured the SIT by calculating the difference between the maximal forces generated by PNS (PNS-SIT) or TMS (TMS-SIT) and the MVC' force. PTs were determined as the maximal force produced by PNS when at rest, at the end of each block. We computed Peripheral (PF), Central (CF), and Supraspinal fatigue (SF) values (i.e., changes in M1 output) based on changes in PNS- and TMS-evoked SIT amplitude and PT amplitude across blocks as described in a previous study[7]. Precisely, PF was quantified using the formula: PF = (1 − (PT' / PTpre)) × 100, which measures the percentage decline in force from baseline. We determined central activation (CA) by normalizing the PNS-SIT' to PT' using the formula: CA = (1 − PNS-SIT'/PT') × 100. The CF was then calculated based on the progressive decline in CA using: CF = [(1 − (CA'/CApre)) × 100], which measures the reduction in CA over time. We computed each block's estimated resting twitch (ERT) as the y-intercept from a least-squares linear regression of TMS-SIT' against MVC' percentage. Supraspinal activation (SA) was then evaluated by normalizing TMS-SIT' at 100% MVC against ERT' using the formula: SA = (1 − (TMS-SIT100'/ ERT')) x 100. The SF is then quantified as the progressive decline in SA, calculated with: SF = [(1 − (SA'/SAfirst block)) × 100. The average PF, CF, and SF values across the last quartile of completed blocks were calculated to deduce each participant's task failure value.

TMS-EEG signals were pre-processed in MATLAB (2020b) with custom scripts using EEGLAB[24] and TESA[25] toolboxes following the same steps and procedures previously described[7]. Continuous EEG signals were epoched from − 1.4 to 1.4 s around the TMS pulse and demeaned. Noisy epochs were removed by visual inspection. The stimulation artifact was eliminated by cutting the signal from 5 ms before to 10 ms after TMS. Data was downsampled to 1000 Hz, and TMS-associated decay and muscle artifacts were removed using independent component analysis (ICA). The removed signals were interpolated, and the epochs were filtered (1–100 Hz bandpass, 48–52 Hz bandstop). Epochs were shortened to − 1.2 to 1.2 s, and a second round of ICA removed residual artifacts.

Clean TMS-EEG epochs were imported into Brainstorm for source reconstruction. Forward modeling was done with the symmetric boundary element method. Cleaned TMS-EEG epochs were imported into Brainstorm (https://neuroimage.usc.edu/brainstorm) for source-level reconstruction using an MRI template (ICBM152) and a 32-channel EEG cap aligned to anatomical templates. Noise covariance was estimated from pre-TMS baselines (− 600 to − 100 ms). Inverse modeling used the Dipole Modeling method, producing a current density time series of TMS-evoked EEG activity for each cortical vertex. Averages were computed using norm data, and differences were computed on z-scored norm data (− 600 to − 100 ms baseline). M1 effective connectivity was quantified by analyzing source-reconstructed TMS-evoked activity within an area-specific time of interest (TOI) spanning 15–60 ms post-TMS[26]. We calculated the grand-average source-reconstructed TMS-evoked activity across all patients and conditions (pre- and post-fatigue) and we defined a region of interest (ROI) as the area where current density values exceeded the 95th percentile of the distribution during the TOI.

Statistical analysis

The dependent variables used for statistical analysis included clinical variables MFIS, BDI-II, and EDSS, as well as neurophysiological variables RMT, PNS intensity, TMS-SIT intensity, MVC, PF, CF, SF, and TMS-evoked source activity. We compared median values of clinical and neurophysiological measures (except TMS-evoked source activity) between T0 and T1 using Wilcoxon signed-rank tests implemented in IBM SPSS (v25). Source-reconstructed TMS-evoked activity pre-fatigue was compared between T0 and T1 with a paired t-test-based permutation analysis using MATLAB. Similarly, TMS-evoked source activity pre- and post-fatigue was compared using separate paired t-test-based permutation analyses for T0 and T1. Differences between pre- and post-fatigue

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(post–pre) were computed using time series z-scored to the baseline time window and compared between T1 and T0 with an additional paired t-test-based permutation analysis to assess the effect of wearing-off. All comparisons were False discovery rate (FDR)-corrected for multiple time points. Spearman's rank-order correlations were conducted to assess the relationships between clinical and neurophysiological variables. A p-value < 0.05 was considered significant. Data normality was assessed using the Shapiro–Wilk test.

Results

Among the 45 patients, three dropped out due to an inability to adapt their force to the different levels required by the motor task. Forty-two patients completed all the clinical and neurophysiological assessments (average age 36.9 ± 8.4 years, 29 females, median disease duration 102 months, range 15–312 months). No adverse events were reported in any study procedure. No clinical relapses were reported during the study. Clinical and neurophysiological variables did not follow a normal distribution. MFIS's and BDI-II's median values were significantly lower at T1 than at T0 (26.0 vs. 35.5, 9.5 vs 13.0; see Table 1 for details). No patient showed changes in EDSS between T0 and T1 (2 vs 2; see Table 1) (Fig. 2).

Neuromuscular assessment showed that median values of Motor fatigue index, Supraspinal fatigue, and Central fatigue were significantly higher at T0 than at T1 (− 0.06 vs. − 0.29, 50.04% vs. 41.57%, 34.46% vs. 24.74% respectively). In contrast, peripheral fatigue median values were not (53.04% vs. 55.09%). Stimulation intensities median values did not statistically differ between T0 and T1 (RMT 72.5 vs. 72 MSO%, PNS 35 vs. 35 mA, TMS SIT 65 vs. 65 MSO%) (Table 2) (Fig. 3).

TMS-EEG data from four patients were excluded due to excessive artifacts. The grand-averaged TMS-evoked potentials (TEPs) in our region and time of interest exhibited, both at T0 and T1, two components peaking between 25 and 30 ms and between 40 and 50 ms, consistent with the well-known P30 and N45 components resulting from M1 stimulation[27] (Fig. 4a). The source-reconstructed TMS-evoked activity peak in the TOI

	MFIS	BDI	EDSS
Wearing-off (T0)	35.5 (5–67)	13 (0–19)	2 (0–6)
Post-infusion (T1)	26.0 (1–62)	9.5 (0–24)	2 (0–6)
Z (p)	− 3.31 (0.001)	0 (1.000)	− 3.737 (< 0.001)

Table 1. Clinical scores at wearing-off (T0) vs. post-natalizumab infusion (T1). [median (range)] *MFIS* Modified Fatigue Impact Scale, *EDSS* Expanded Disability Status Scale, *BDI-II* Beck Depression Inventory-II. *Z* Wilcoxon signed-rank test. *P* p-value.

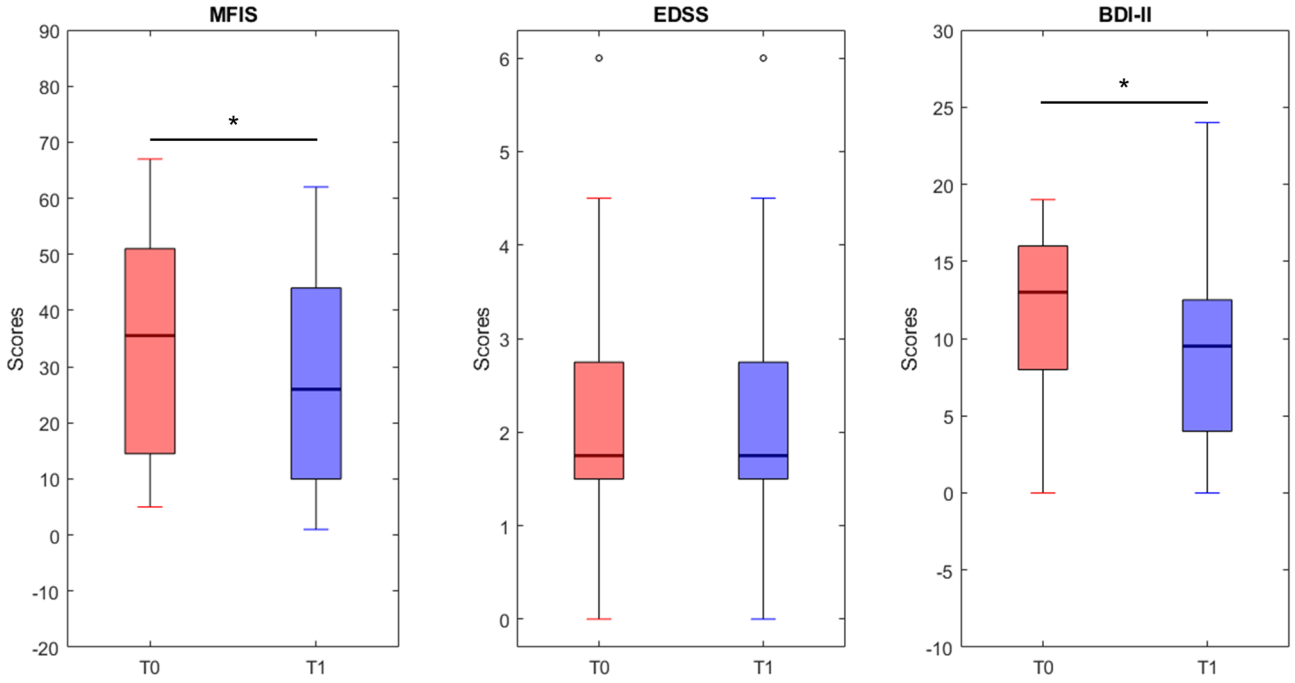

Figure 2. Clinical scores at wearing-off (T0) vs. post-infusion (T1). Boxplots of clinical measures measured before (T0, red) and after (T1, blue) natalizumab infusion. Lines: median values. Error bars: interquartile range. Circles: outliers. *Significant difference with a p < 0.05. *BDI-II* Becks Depression Inventory – Second Edition, *EDSS* Expanded Disability Status Scale, *MFIS* Modified Fatigue Impact Score.

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	RMT	PNS intensity	TMS-SIT intensity	Motor fatigue	MVC*	Peripheral Fatigue*	Central fatigue*	Supraspinal Fatigue*
T0	72,5 (47–90)	35 (20–60)	65 (45–80)	−0.06 (−1.43–2.45)	63.89 (32.83–101.91)	53.04 (4.69–76.54)	34.46 (3.84–71.38)	50.04 (4.2–157.43)
T1	72,0 (45–95)	35 (20–55)	65 (45–85)	−0.29 (−1.16–2.80)	64.10 (41.28–82.18)	55.09 (0.00–84.62)	24.74 (0.08–81.22)	41.57 (0.51–84.49)
Z (p)	−0.719 (0.472)	−1.086 (0.277)	−0.33 (0.741)	−2.917 (0.004)	−0.223 (0.823)	−0.202 (0.840)	−2.621 (0.009)	−2.621 (0.009)

Table 2. Neuromuscular assessment at wearing-off (T0) vs. post-natalizumab infusion (T1). [median (range)] *MFIS* Modified Fatigue Impact Scale, *EDSS* Expanded Disability Status Scale, *BDI-II* Beck Depression Inventory-II. *PNS* peripheral nerve stimulation, *RMT* rest motor threshold, *TMS-SIT* transcranial magnetic stimulation-evoked superimposed-twitch, *MVC* maximal voluntary contraction, *TEP delta* TMS-evoked cortical activation differences between pre- and post-fatigue. *Values at task failure. *Z* Wilcoxon signed-rank test. *P* p-value.

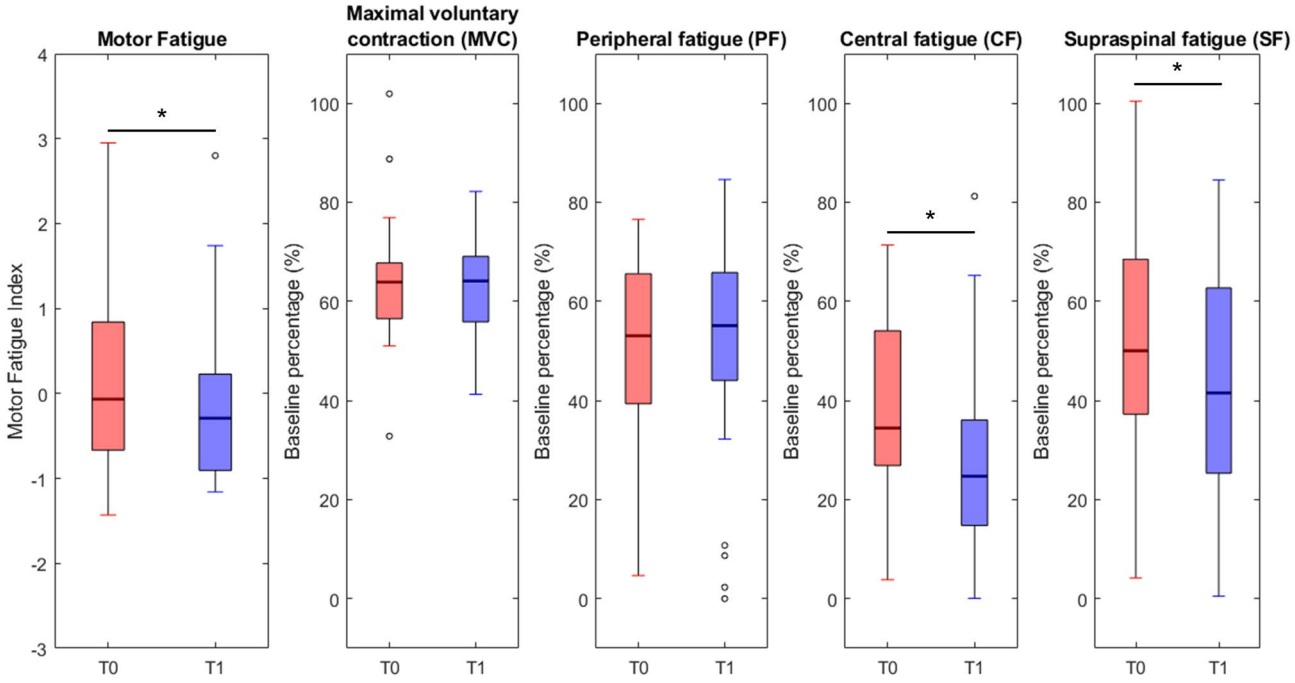

Figure 3. Neurophysiological measures at wearing-off (T0) vs. post-infusion (T1). Boxplots of neurophysiological measures measured before (T0, red) and after (T1, blue) natalizumab infusion. Lines: median values. Error bars: interquartile range. Circles: outliers. *Significant difference with a $p < 0.05$.

predominantly localized to the hand area of the left M1 and the adjacent primary somatosensory cortex, consistent with propagation within the SMN (TMS-evoked SMN activity) (Fig. 4b). We found no significant differences in TMS-evoked SMN activity before the fatigue-inducing task (i.e., pre-fatigue) between the two time points: at T0 (wearing-off) and T1 (post-natalizumab assessment). We found a different effect of the fatigue-inducing task on TMS-evoked SMN activity between T0 and T1. Specifically, at wearing-off (T0), we found no significant difference in TMS-evoked SMN activity when comparing before and after the fatigue-inducing task (pre- vs. post-fatigue) (Fig. 4b,c, left). Conversely, after the administration of natalizumab (T1), there was a significant decrease in TMS-evoked SMN activity at post-fatigue compared to pre-fatigue (Fig. 4b,c, right). This decrease was observed in two distinct time intervals: from 15–23 ms and 35–58 ms post-TMS ($p < 0.05$). To confirm the difference in the effect of fatigue at T1 compared to T0, we examined the change in TMS-evoked SMN activity between the pre- and post-fatigue states (post–pre). Our analysis revealed that at T1, there was a significantly greater decrease in TMS-evoked SMN activity between pre- and post-fatigue states when compared to T0 in almost identical two distinct time intervals: from 15 to 25 ms and from 32 to 60 ms post-TMS ($p < 0.05$), (Table 2), (Fig. 4d).

Given the observed differences in BDI-II score between T0 and T1, we explored potential correlations with changes in the neurophysiological measures (T1–T0). For correlation analysis, we averaged the current source density of TMS-evoked SMN activity across the 32 to 60 ms interval, thus excluding the 15–25 ms interval to limit contaminations due to residual TMS-evoked muscle activity[28]. We found that changes in BDI-II score (T1–T0) showed a significant direct correlation with changes in MFIS ($r_s = 0.39$, $p = 0.013$) but no significant correlation with changes in motor fatigue ($r_s = 0.21$, $p = 0.196$), SF (i.e. M1 output) ($r_s = 0.20$, $p = 0.224$) and fatigue-related modulation of TMS-evoked SMN activity (i.e. M1 effective connectivity) ($r_s = -0.05$, $p = 0.771$).

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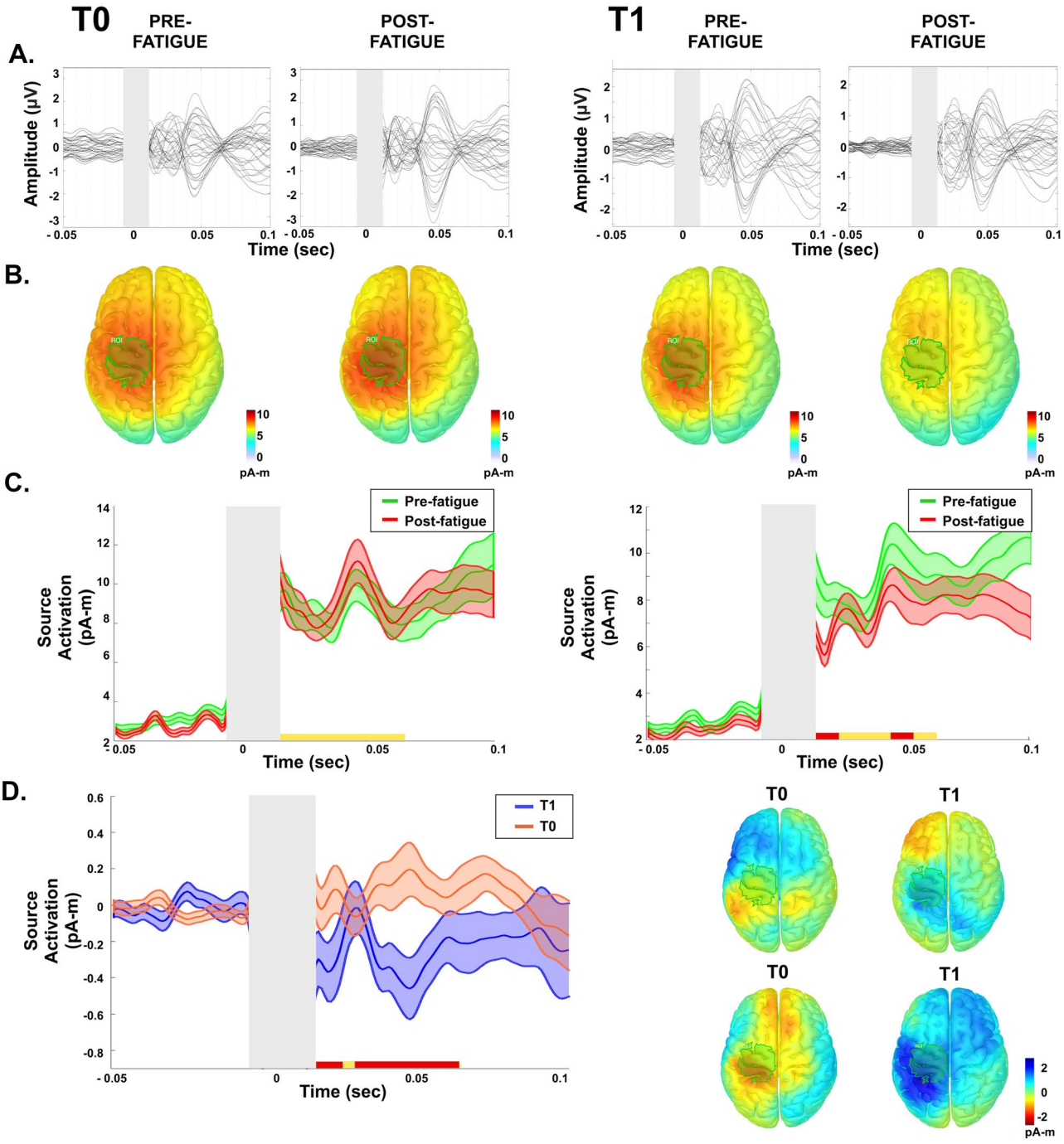

Figure 4. Fatigue-related modulation of TMS-evoked cortical activation at wearing-off (T0) vs. post-infusion (T1). **a** Grand average TEPs butterfly plots from left M1 stimulation, common average reference; greybars: interpolated signals. **b** Brain topographies of grand average TMS-evoked cortical source activation within the time window of interest (TOI) ranging from 15 to 60 ms; green-shaded areas: Region Of Interest (ROI) within the sensorimotor network. **c** Time series of grand average TMS-evoked cortical activation's time series in the ROI; yellow bar: TOI; red bars: time intervals showing significant differences between pre- and post-fatigue. **d** (Left) Time series of grand average TMS-evoked cortical activation differences within the ROI between pre- and post-fatigue (post minus pre). Red bars: time intervals showing significant differences between T0 and T1. (Right) Brain topographies of grand average TMS-evoked cortical activation differences between pre- and post-fatigue. First row: brain topography at 22 ms; second row: brain topography at 45 ms.

Discussion

In this study, we found a worsening of motor fatigue, objectively evaluated through neuromuscular assessment,

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during the "wearing-off" phenomenon compared to the period following natalizumab infusion. We also demonstrated that the increased motor fatigue observed during the wearing-off period, compared to post-natalizumab infusion, was due to central and supraspinal mechanisms, as evaluated during the fatiguing task. Also, patients at wearing-off showed a reduced task-related modulation of M1 effective connectivity within the sensorimotor network as assessed through TMS-EEG. Overall, the changes in neurophysiological parameters suggest that wearing-off is characterized by increased motor fatigue due to an impaired M1 output and reduced task-related modulation of M1 effective connectivity within the SMN[7].

We took several precautions to avoid confounding factors. We excluded patients with severity of depressive symptoms that could potentially influence fatigue symptoms and neurophysiological assessments. We also excluded patients with clinical evidence of motor or sensory impairment in the upper limb involved in the task. To further control for variability, we focused on patients who were in chronic treatment and exhibited stable disease activity, as this would allow for a more accurate assessment of the effects of wearing-off on fatigue symptoms, thus eliminating the confounding effects of disease exacerbations or treatment initiation. Also, we only studied right-handed subjects to control for hemispheric dominance, as left-handed individuals often show bilateral activation of the sensorimotor network during unilateral tasks[29], which could complicate the analysis of network alterations induced by motor fatigue.

The neuromuscular assessment showed a higher motor fatigue index at a wearing-off stage than the post-natalizumab infusion. This shows that the subjective feeling of fatigue reported by the patients during the wearing-off period is associated with an objective increase in motor fatigue, as measured by the motor fatigue index. We found similar peripheral fatigue levels at wearing-off and after natalizumab infusion, suggesting that fatigue during the wearing-off is not due to neuromuscular transmission or muscle contraction mechanisms. The higher central fatigue component we found at wearing-off indicates a possible modulation in the descending motor drive, potentially linked to altered corticospinal transmission or suboptimal M1 output[6]. The concurrent observation of higher supraspinal fatigue at wearing-off compared to post-natalizumab suggests that wearing-off-related motor fatigue is mainly due to defective M1 output[19,20]. Our previous study suggested that motor fatigue in MS compared to healthy controls is mainly driven by supraspinal mechanisms due to failure in activating pyramidal tract neurons at the M1 level[7]. The defective M1 output supports the idea that the wearing-off has similar mechanisms to those responsible for motor fatigue pathophysiology in MS.

TMS-EEG further clarified the mechanisms involved in the decreased M1 output during wearing-off. TMS-evoked source activity is thought to reflect both the excitability and the effective connectivity of the stimulated area within its functional network [7,8,23,26,27,30,31]. The present study's findings indicate that MS patients experiencing wearing-off symptoms do not exhibit the significant post-fatigue modulation of TMS-evoked SMN activity previously observed in healthy subjects[7]. However, this modulation of SMN activity is restored following natalizumab infusion. Our findings indicated that wearing-off affected TMS-evoked SMN activity at two distinct intervals at 20 and 45 ms post-TMS. We focused exclusively on the 45 ms interval to avoid the confounding effects of early residual muscle artifacts[23,28], because the 45 ms interval corresponds to well-defined N45 TEP component[8,26,27], and finally, because we previously described abnormal fatigue-induced modulation of TEPs at this interval in MS[7]. Therefore, the present study suggests that wearing-off is associated with an exacerbation of the mechanisms causing motor fatigue in MS, i.e., abnormal modulation of M1 effective connectivity in response to a motor task, and that natalizumab significantly improves them. The post-natalizumab improvement in motor fatigue, paralleled by M1 output and effective connectivity improvements, suggests that their worsening at wearing-off reflects dysfunction rather than structural changes within the SMN.

Confirming previous clinical observations[9,32], we observed worse fatigue symptoms, as measured by the 21-item MFI, and depressive symptoms, as indicated by the BDI-II, during the wearing-off period. These changes were observed independently from clinical relapses or EDSS changes and confirm natalizumab's multi-faceted effects independent of its control on disease activity. Patients enrolled in this study had no clinically relevant depression. However, the correlation observed between BDI-II and MFIS scores suggests a link between fatigue and depression symptoms. On the other hand, the absence of correlation between changes in BDI-II and objective measures of motor fatigue suggests that depressive symptoms do not explain the neurophysiological changes we found. The lack of change in EDSS was anticipated due to our study's relatively short observation period and the fact that participants were clinically stable, having been on chronic natalizumab treatment.

We acknowledge some limitations. Our patient selection was based on self-reported wearing-off fatigue symptoms, which introduces a potential subjective bias and may not capture the full complexity of the wearing-off phenomenon. However, our neuromuscular assessments demonstrated that the patients we selected did indeed experience a significant, objective worsening of motor fatigue at wearing-off. Also, we excluded patients with an EDSS of more than 6.5, considering that the task might have been too strenuous for such a population. Nonetheless, this limits the generalizability of our findings to more severe patients, where other neural mechanisms might come into play in the pathophysiology of motor fatigue, and which are still too often excluded from MS studies due to fear of little compliance. Another limitation of our study is the absence of lesion load data, which could have influenced our understanding of cortical responses. While our choice of excluding patients with clinically evident sensory and motor deficits reduced the potential lesion load within the sensorimotor network, we cannot discount the possibility of clinically silent lesions that might still modulate the network's response to fatigue. This study focuses solely on motor components, and we did not assess other possible components of the wearing-off. Although we attempted to control for potential confounding factors, additional variables, such as participants' physical activity levels, sleep quality, or other medication use, could have influenced the results.

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Conclusions

In conclusion, our findings suggest that the mechanisms of motor fatigue during wearing-off are similar to those underlying MS-related motor fatigue. Specifically, the wearing-off-related fatigue in MS is caused by an impaired M1 output and altered task-induced modulation of M1 effective connectivity within the SMN[7]. Given the improvement of these abnormalities following natalizumab infusion, motor fatigue in MS may reflect reversible inflammation-derived changes in neural transmission within the SMN. Our results suggest that natalizumab's ability to reduce motor fatigue is likely due to its influence on cortical processes involved in the underlying mechanisms of motor fatigue. Although our findings apply primarily to MS patients receiving natalizumab, we believe they provide valuable insights into the broader mechanisms of MS-related fatigue. Further research is essential to generalize our findings, particularly in patients under different treatments that exhibit a wearing-off effect[33]. Finally, the objective assessment of motor fatigue we developed will be helpful in evaluating the effects of other disease-modifying therapies on motor fatigue.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

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Author contributions

G.L.: Conceptualization, Methodology, Investigation, Writing—original draft, Formal analysis. M.M.: Visualization, Investigation, Formal analysis, Writing—original draft. D.V.: Visualization, Data curation, Investigation, Software, Writing—original draft. M.T.: Data curation, Investigation, Resources. A.I.: Investigation, Resources, Data curation. F.C.: Investigation, Formal analysis, Data curation. G.F.: Resources, Data curation, Writing—review & editing. L.M.: Resources, Investigation, Data curation. D.B.: Supervision, Conceptualization, Writing—review & editing. C.P.: Supervision, Conceptualization, Writing—review & editing, Project administration, Funding acquisition. A.B.: Supervision, Conceptualization, Methodology, Writing—review & editing, Funding acquisition. A.C.: Supervision, Conceptualization, Methodology, Writing—review & editing, Project administration, Funding acquisition. All authors reviewed the manuscript and have approved the final article.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to G.L.

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