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Original article

Contribution of thalamus-cortex network and cortical habituation mechanisms in multiple sclerosis

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ABSTRACT

Objective: Fatigue is one of the most prevalent and debilitating symptoms in individuals with multiple sclerosis (MS). We recorded somatosensory high-frequency oscillatory (HFO) thalamocortical activity in a group of MS patients without history of optic neuritis (ON). Furthermore, we examined if, like what was previously observed in response to visual stimuli, patients exhibit diminished amplitude habituation, and whether this may further exacerbate fatigue in individuals with MS.

Methods: Twenty patients diagnosed with relapsing-remitting MS were prospectively enrolled. The MS cohorts were compared with a cohort of 20 healthy volunteers (HV). Fatigue Severity Scale (FSS) was employed to evaluate the trait levels of reported fatigue. We assessed the N20 somatosensory evoked potentials (SSEPs) parameters and N20-P25 amplitude habituation, and, following the application of a band-pass filter (450–750 Hz), we evaluated electrophysiological parameters of pre- and post-synaptic HFOs.

Results: The latency and amplitude of the N20 SSEP and its habituation do not exhibit significant differences between MS patients and HVs. MS patients exhibited delayed latency of negative oscillatory maximum and reduced maximum peak-to-peak amplitude of the pre-synaptic HFOs (all $p < 0.01$). In patients, post-synaptic HFOs showed a significant delayed latency and a trend towards a reduction in the maximal peak-to-peak amplitude. The pre-synaptic HFO latency of the negative oscillatory maximum, reflecting thalamocortical activity, shows a correlation with the FSS in MS patients ($r = 0.522$, $p = 0.018$). No significant ophthalmological anomalies were identified.

Conclusions: Our data revealed a significant reduction and slowing of somatosensory thalamocortical network activity in MS patients without history of ON. Furthermore, our findings showed that fatigue levels may be affected by slowed thalamocortical activity, but not by habituation of cortical responses.

Significance: Our findings suggest a potential role of the thalamo-cortical network on the genesis of MS-related fatigue.

Abbreviations: AChE, Acetylcholine; EDSS, Expanded disability status scale; FSS, Fatigue Severity Scale; GABA, gamma-aminobutyric acid; HV, Healthy volunteer; HFO, High-frequency oscillation; KD, Ketogenic diet; MRI, Magnetic resonance imaging; MS, multiple sclerosis; SSEP, somatosensory evoked potential; VEP, visual evoked potential.

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1. Introduction

Multiple sclerosis (MS) is the predominant neurological autoimmune disorder and the primary cause of neurological disability among young individuals, characterized by demyelination, axonal loss, and neurodegeneration (Filippi et al., 2018).

Fatigue, characterized by an intense deficiency of physical and/or mental energy, is recognized as one of the most common and disabling symptoms among people with MS (Bakshi, 2003). In the past two decades, numerous studies have concentrated on the pathophysiology of fatigue in MS, with mounting evidence indicating that a dysfunction in the cortico-subcortical pathway, particularly involving the basal ganglia and the thalamus, may constitute its primary pathogenic substrate (Capone et al., 2020a; Engström et al., 2013; Hidalgo de la Cruz et al., 2018; Rocca et al., 2014). Structural damage in the basal ganglia and its decreased functional connectivity with motor cortex or frontal areas, in close relation to cognitive and subjective fatigue, have been reported (Calabrese et al., 2010; Finke et al., 2015). In addition, various authors have reported structural atrophy in the thalamo-cortical pathways and alterations in resting-state MRI functional connectivity of MS patients between distinct thalamic nuclei and brain regions associated with cognitive and sensorimotor functions, as well as cognitive fatigue, by employing different fatigue measurement scales, like the Fatigue Severity Scale (FSS) (Capone et al., 2020a; Hidalgo de la Cruz et al., 2018). The deviations in functional connectivity have been regarded as maladaptive plastic mechanisms attempting to compensate for fatigue, ultimately without success. Response habituation to stereotyped stimulus repetition, a form of plastic learning and a protective mechanism designed to mitigate information overload for the brain, was observed to be impaired in MS patients without previous optic neuropathy (ON), as evidenced by pattern electroretinogram (Fadda et al., 2013) and visual evoked potentials (VEPs) (Bir et al., 2013). The absence of habituation in MS may enhance cerebral energy requirements and neuronal strain, leading to the excessive buildup of metabolites like lactate, all of which could further adversely affect cognitive fatigue in individuals with MS.

In humans, thalamo-cortical drive can be assessed, non-invasively, by examining the high-frequency oscillations (HFOs) (about 600 Hz) present in the broadband somatosensory evoked potentials (SSEPs) induced by median nerve electrical stimulation at the wrist (Curio, 2000). In MS patients, somatosensory HFOs were identified as a sensitive method for detecting disrupted signal propagation resulting from demyelination and axonal injury (Gobbelé et al., 2003). Capone et al. (Capone et al., 2020b, p. 202) showed that fatiguing activities preferentially affect several characteristics of HFOs that indicate the functionality of the thalamo-cortical system. Researchers discovered that in MS patients, repeated exertions resulted in diminished strength performance and a reduction in the overall area of HFOs, primarily attributable to the attenuation of the early thalamo-cortical component (Capone et al., 2020b).

To further understand the role of the cortico-subcortical substrates in the pathogenesis of MS-related fatigue, we recorded HFO thalamo-cortical activity and SSEP habituation in a cohort of MS patients without history of ON compared to a group of healthy controls. We hypothesized that the hyperactivity of the somatosensory system measured using habituation recordings, and the abnormal function of the thalamo-cortical loop may be contributing factors to the degree of fatigue in MS patients without previous ON.

2. Material and methods

2.1. Subjects

Twenty patients diagnosed with clinically definite relapsing-remitting MS according to the Thompson criteria (Thompson et al., 2018) were prospectively recruited from our outpatients clinic. Inclusion criteria were age 18–65 years and not having experienced a relapse within 1 month of testing. Patients were excluded if they had been receiving medication on a regular basis in the 3 months preceding the screening visit, including corticosteroids, antibiotics, benzodiazepines, antidepressants, but not oral contraceptive medication (disease-modifying drugs for MS were allowed, with 10 patients under dimethyl fumarate, six under peginterferone beta-1a, three under fingolimod, and one under glatiramer acetate), had a history of diabetes or other metabolic or autoimmune disease other than MS, peripheral nerve pathology, systemic hypertension, or had any type of primary or secondary headache. Additional exclusion criteria were the presence of ON in both eyes. The absence of ON was established based on: full high-contrast Best Corrected Visual Acuity (BCVA), absence of relative afferent pupillary reflex, absence of visual field defects (Humphrey Field Analyzer SITA standard 30–2 strategy), normal colour vision (Ishihara pseudoisochromatic plates), absence of ocular pain during eye movements, absence of signal abnormalities at the level of optic nerve on MRI, normal VEP responses (see below). All participants received comprehensive information about the study after which we acquired written informed consent before the start of the recording session. The local ethics review board approved the study, and the study adhered to the Declaration of Helsinki.

Prior to electrophysiological testing, the Fatigue Severity Scale (FSS) (Krupp et al., 1989) was used to assess the trait levels of perceived fatigability. As a control group, 20 age- and sex-matched healthy volunteers (HVs) without personal history of any health conditions or first or second-degree relatives' history of MS or other neurological disorders, including migraine, were recruited and randomly recorded between patients. Both the patients and the controls were recorded in the same laboratory. The data in the present study were collected between 2023 and 2024. In female participants, experiments were conducted mid-menstrual cycle (11.8 $\pm$ 3.9 days from menstruation onset in HVs, 13.8 $\pm$ 3.8 in patients with MS) to avoid hormonal interference. All participants were right-hand dominant.

2.2. Acquisition of SSEPs

SSEPs were recorded stimulating the median nerve at the right wrist. The experiment was performed by two investigators (L.C. & B.P.) in the same laboratory. We did not retain a record of how many and which participants of the study were recorded by each investigator. Stimuli were delivered at a rate of 4.4 Hz using monophasic rectangular pulses (0.2 ms width, proximal cathode), at 1.2 times the intensity for the motor threshold. The sensory thresholds were not recorded. The first electrode was placed over the scalp, 2 cm posterior to the C3 position of the International 10–20 system. The second and third electrodes were referenced to the Fz position of the International 10–20 system and recorded with a band electrode placed around the right forearm as a ground lead. A Digitimer™ D360 amplifier (Digitimer Ltd, Hertfordshire, UK) (band-pass 0.05–2500 Hz, 1000 $\times$ gain) was used for amplification, and a CED™ power 1401–4 device (Cambridge Electronic Design Ltd, Cambridge, UK) was used to record SSEP signals.

The recording sessions took place between 2 and 6 pm. Participants were instructed to keep their eyes open throughout the recording session in a comfortable seated position in an illuminated room. They were asked to focus their attention on the thumb movements induced by the stimuli without looking at thumb. Six-hundred sweeps of 50 ms duration (sampling rate, 5 kHz) were collected. GC analyzed all recordings off-line, blinded to the subjects' diagnosis, with the Signal™ software package version 4.10 (CED Ltd). Artifacts exceeding 90% of the analog-to-digital converter (ADC) range (confirmed by visual inspection) were automatically rejected using the Signal™ artifact rejection tool. A correction for DC drift artifacts was applied to the evoked signal offline, and we removed eye movements and blink artifacts.

2.3. Low-frequency (LF-) SSEPs

A digital filter between 0 and 450 Hz was applied to the signal, and we identified the various SSEP components (N20 and P25) in accordance with their respective latencies. Peak-to-peak amplitudes were calculated of the cortical N20–P25.

Since in previous studies in healthy subjects a significant reduction (habituation) in the amplitude of the SSEP could already be observed from the second block of 100 responses onward (Coppola et al., 2013; Ozkul and Uckardes, 2002), we partitioned the first 300 evoked responses into three consecutive blocks of 100 responses. Each block was averaged offline ("block averages") and analyzed for N20–P25 amplitudes. Habituation was expressed as the slope of the linear regression line for the second and third blocks (Coppola et al., 2013). We analyzed N20–P25 peak-to-peak amplitudes for each block. Habituation was expressed as the slope of the linear regression in the N20–P25 amplitude between blocks 1 and 2 and between blocks 1 and 3.

2.4. High-frequency SSEPs

Using a method described elsewhere (Abagnale et al., 2024), digital zero-phase shift band-pass filtering between 450 and 750 Hz (Barlett-Hanning window, 51 filter coefficients) was applied off-line on median nerve SSEPs elicited by median to extract the HFOs embedded in the parietal N20 SSEP component. In most recordings, we were able to identify two separate bursts of HFOs: an early pre-synaptic burst within the latency interval of the ascending slope of the conventional N20 SSEP component and a late post-synaptic burst in the time interval of the descending slope of N20, sometimes extending into the ascending slope of the N33 peak. In general, the frequency of the oscillations was higher in the 1st than in the 2nd burst and in between the pre- and post- synaptic bursts there was a clear frequency and amplitude decrease, which allowed separating the two bursts. In recordings in which a clear distinction between the two components was not possible, we considered HFOs occurring before the N20 peak as pre-synaptic bursts and those after the N20 peak as post-synaptic bursts.

Latency of the negative oscillatory maximum, intra-burst frequency, number of negative peaks, and maximum peak-to-peak amplitude were measured after the stimulus artifact was eliminated for each the two HFO bursts (Abagnale et al., 2024).

2.5. Visual evoked potentials recordings

Monocular pattern VEP were recorded by using the Retimax Advanced Plus system (CSO, Florence, Italy). Visual stimuli consisted of high-contrast (80%) checkerboard patterns, subtending either 60 min or 15 min of arc, presented on a monitor (mean luminance of 110 cd/m^2, reversal in contrast at the rate of 2 reversal per seconds, viewing distance of 114 cm), according to the International Society of Clinical Electrophysiology of Vision Standards (Odom et al., 2016). VEPs were obtained from the scalp with electrodes placed at Oz (active electrode) and Fz (reference electrode) according to the International 10–20 system. A ground electrode was positioned on the right forearm. P100 implicit time and N75-P100 amplitude of 15′ and 60′ VEP responses were measured. Each recording session included a minimum of two and a maximum of six repeated acquisitions to verify waveform reproducibility (Parisi et al., 2021).

2.6. Statistical analysis

All analyses were performed using Statistica for Windows (StatSoft Inc., Tulsa, OK, USA) version 8.0. An independent sample *t*-test was used to compare normally distributed data between individuals with MS and HV, otherwise, the Mann-Whitney U test was used for non-normally distributed and the Welch's *t*-test for normally distributed data but with violation of the assumption of equal variances (significant Levene's test = $p < 0.05$). For all the analyses, a p-value < 0.05 was considered statistically significant. Bonferroni method was used to correct for multiple comparisons. We used Pearson's correlation coefficient and corrected for multiple comparisons to assess the correlation between significant electrophysiological data (Latency and amplitude of the negative oscillatory maximum of pre-HFO and post-HFO) and expanded disability status scale (EDSS) and FSS. The significance threshold was set at $p < 0.05$.

3. Results

Demographic and clinical features of MS patients and HVs are summarized in Table 1.

3.1. SSEPs, basic neurophysiological parameters

All individuals had complete and valid SSEP recordings. Table 2 lists baseline electrophysiological parameters. Motor thresholds were similar between groups (HV vs. MS $t = 1.92$, $p = 0.06$). There were no significant changes between groups in N20 latency or peak-to-peak amplitude ($p > 0.05$) (Table 2).

3.2. SSEPs, response habituation

The amplitudes of the three blocks were consistent among the groups ($p > 0.05$, Table 2, Fig. 1). In both HVs and patients with MS, the N20–P25 amplitude exhibited a decline from block 1 to block 2 and from block 1 to block 3, indicating normal habituation (slope from block 1 to block 2: −0.307 in HVs vs. −0.138 in MS, $t = 0.899$, $p = 0.375$; slope from block 1 to block 3: −0.122 in HVs vs. −0.091 in MS, $t = 0.224$, $p = 0.824$, refer to Table 2).

3.3. SSEPs, high-frequency oscillations (HFOs)

In the MS group, the latencies of the negative oscillatory maximum for pre- and post-synaptic HFOs were significantly prolonged ($t = 3.08$, $p = 0.004$ for pre-synaptic; $U = 97$, $p = 0.003$ for post-synaptic, Fig. 2). The maximum peak-to-peak amplitude of the pre-synaptic HFO was significantly diminished ($t = 4.39$, $p < 0.001$) in the MS group compared to HVs; the significance of the maximum peak-to-peak amplitude of the post-synaptic HFOs did not survive Bonferroni correction ($t = 2.49$, $p = 0.017$) (Table 3). Additionally, the intra-burst frequency of the post-synaptic HFOs was significantly diminished in the MS group compared to HVs ($t = 2.71$, $p = 0.01$ for post-synaptic HFOs). The number of peaks and burst durations did not exhibit significant differences between the groups ($p > 0.05$, Table 3).

3.4. Neuro-ophthalmological features

All neuroophthalmological characteristics of MS patients, encompassing 40 eyes, were comparable to those of HVs (Table 4).

Table 1
Demographic and clinical characteristics of healthy volunteers (HVs) and multiple sclerosis (MS) patients. EDSS: Expanded Disability Status Scale, FSS: Fatigue Severity Score.

	HVs	MS	Statistics
Female (n)	11	13	$X^2 = 0.889$, $p = 0.346$
Age (y)	39.5 ± 10.9	44.4 ± 11.3	$t = 1.43$, $p = 0.159$
Height (cm)	173.0 ± 6.8	170.0 ± 8.3	$t = 1.23$, $p = 0.227$
Weight (Kg)	68.3 ± 8.0	71.8 ± 15.3	$t = 0.918$, $p = 0.364$
Years of illness		9.0 ± 6.4	
EDSS		1.97 ± 1.1	
FSS		32.4 ± 14.2	

Table 2
Parameters of somatosensory-evoked potentials in healthy volunteers (HVs) and multiple sclerosis (MS) patients. Data are showed as means ± SD (- / + 95% confidence limits) and inferential statistics. Block average N20-P25 amplitudes and habituation slopes of somatosensory-evoked potentials in healthy volunteers (HVs) and multiple sclerosis (MS) patients. Data are showed as means ± SD and inferential statistics, including Cohen's d.

	HVs	MS	Statistics
Motor threshold (mA)	8.5 ± 2.6 (7.4 – 9.5)	6.9 ± 2.6 (5.7 – 8.1)	T = 1.92, p = 0.062, d = 0.62
N20 latency (msec)	19.33 ± 1.15 (18.73 – 19.93)	20.19 ± 1.75 (19.50 – 20.84)	t = 1.89, p = 0.069, d = −0.58
N20-P25 amplitude (μV)	1.67 ± 0.59 (1.28 – 2.05)	1.19 ± 1.10 (0.90 – 1.76)	U = 136, p = 0.055, d = 0.55
1st block (μV)	1.80 ± 0.86 (1.36 – 2.24)	1.48 ± 1.12 (1.00 – 1.97)	U = 141, p = 0.281, d = 0.32
2nd block (μV)	1.49 ± 0.59 (1.13 – 1.86)	1.35 ± 1.04 (0.94 – 1.75)	U = 144, p = 0.322, d = 0.17
3rd block (μV)	1.56 ± 0.69 (1.10 – 2.01)	1.30 ± 1.32 (0.80 – 1.81)	U = 127, p = 0.136, d = 0.25
Habituation slope block 1–2	- 0.307 ± 0.64 (−0.56 – −0.05)	- 0.138 ± 0.48 (−0.42 – 0.14)	t = 0.899, p = 0.375, d = −0.30
Habituation slope block 1–3	- 0.122 ± 0.42 (−0.31 – 0.07)	- 0.091 ± 0.07 (−0.30 – 0.12)	t = 0.224, p = 0.824, d = −0.10

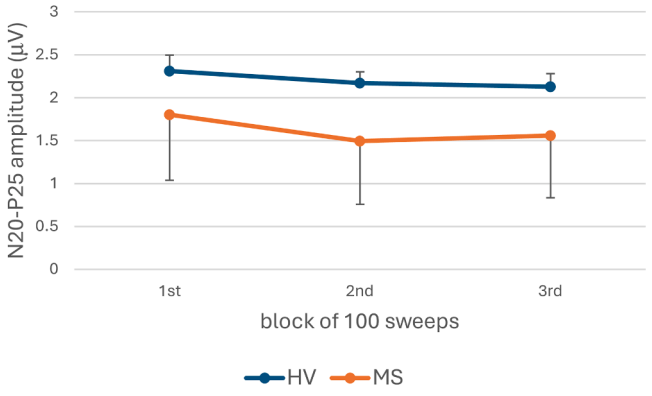

Fig. 1. Amplitudes of the SSEP N20-P25 component (mean ± SEM) over the three sequential blocks of 100 responses in healthy volunteers (HVs) and patients with multiple sclerosis (MS).

3.5. Correlation analyses

In patients with MS, Pearson's correlation tests indicated that the pre-synaptic HFO latency of the negative oscillatory maximum exhibited a significant correlation with the FSS (Pre-HFO r = 0.522, p = 0.018), although the post-synaptic HFO latency just neared significance (Post-HFO r = 0.435, p = 0.055, Fig. 3). No additional association between the FSS and other electrophysiological variables in MS patients was identified. No significant association was identified between the EDSS and electrophysiological measures.

4. Discussion

The primary findings of this study are as follows: a) amplitude and latency of the N20 LF-SSEP do not significantly differ between MS patients and HVs, b) habituation levels in MS patients are comparable to those of HVs, c) the latencies of the negative oscillatory maximum are significantly increased for both pre- and post-synaptic HFOs in MS patients compared to HVs, d) the amplitude of the negative oscillatory maximum is significantly decreased for the pre-synaptic HFO, while tends to decrease for the post-synaptic HFO in MS patients compared to HVs, and e) the pre-synaptic HFO latency of the negative oscillatory maximum, indicative of thalamocortical activity, exhibits a significant correlation with the FSS, in MS patients. The latter finding underscores the significant impact of the thalamo-cortical network in the origin of MS-related fatigue.

The observation that N20 SSEP approaches normalcy despite irregularities in HFO components aligns with several other investigations (Brotherton et al., 2024; Capone et al., 2020b; Gobbelé et al., 2003). This contrasts with other prior conventional SSEP investigations (Chiappa, 1980; Comi et al., 1999; Dubbioso et al., 2022) and may underscore the enhanced sensitivity of HFOs in identifying and evaluating functional changes in the somatosensory pathway of MS patients.

Previous structural and functional neuroimaging and electrophysiological findings may elucidate the anomalies observed in our analysis of HFOs in patients with MS and their connection with fatigue scale scores.

4.1. Modified thalamo-cortical activity in individuals with MS

Brain atrophy is commonly recognised as a prominent histopathological characteristic of multiple sclerosis, as demonstrated by longitudinal (Bakshi et al., 2001; Fox et al., 2000; Ge et al., 2000; Simon, 1999) and cross-sectional MRI investigations (Brex et al., 2001; Losseff et al., 1996; Rudick et al., 1999; Stevenson et al., 1998). Numerous MRI studies (Audoin et al., 2004; Calabrese et al., 2010; Ceccarelli et al., 2008; Dalton et al., 2004; Wylezinska et al., 2003) indicate that the thalamus is one of the initially affected subcortical nuclei in the pathology, even in clinically isolated syndrome, pediatric-onset multiple sclerosis (Aubert-Broche et al., 2011), and radiologically isolated syndrome; consequently, thalamic atrophy is observed across all types of MS (Henry et al., 2008; Ramasamy et al., 2009; Rocca et al., 2007).

Electrophysiological investigations utilising SSEP are among the most effective methods for assessing thalamo-cortical and primary cortical activation. SSEP are more closely linked to impairment than MRI, suggesting they may serve as a predictive biomarker in conjunction with MRI (Wuschek et al., 2023). Animal models have demonstrated that HFOs inside the standard SSEPs serve as electrophysiological indicators of cortical spike bursts (Baker et al., 2003). The high-frequency bursts overlaying the traditional broad-band somatosensory response are believed to represent rapidly recurring population spikes in thalamo-cortical axons, along with supplementary wavelet generators inside the grey matter of S1 (Curio, 2000). Pre-synaptic burst HFOs signify action potentials from the terminal tract of thalamo-cortical axons, whereas post-synaptic bursts arise from the activity of intracortical GABAergic interneurons located in the somatosensory cortex (Coppola et al., 2005; Ozaki and Hashimoto, 2005). Our MS patients exhibited diminished amplitude and a delayed peak in the pre-synaptic burst of HFOs compared to HVs. Moreover, in patients, the post-synaptic cortical component exhibited increased peak latency, a notable decrease in intra-burst frequency, and a trend for amplitude reduction relative to healthy individuals.

Consistent with prior research, aberrant HFOs and SSEP N20 may result from demyelination and axonal injury, respectively, attributable to the neurodegenerative characteristics of MS pathology (Hardmeier et al., 2022; Waxman, 2006).

Our observations of diminished amplitudes accompanied by delayed conduction of HFOs, alongside normal-to-reduced N20, may result from temporal dispersion of impulse propagation attributable to demyelinating lesions, with minimal axonal involvement (Gobbelé et al., 2003).

Pharmacological studies show that increasing GABA or acetylcholine (ACh) bioavailability can enhance HFO amplitude and frequency (Haueisen et al., 2000; Restuccia et al., 2003). In MS, this biochemical balance is often disrupted. Reduced GABA levels in the somatosensory cortex of MS patients have been linked to altered network connectivity and impaired inhibitory control (Droby et al., 2020). Furthermore, cholinergic dysfunction in MS appears to correlate with the underlying neuro-inflammatory process (Gatta et al., 2020). This neurochemical imbalance is further evidenced by studies on short-latency afferent

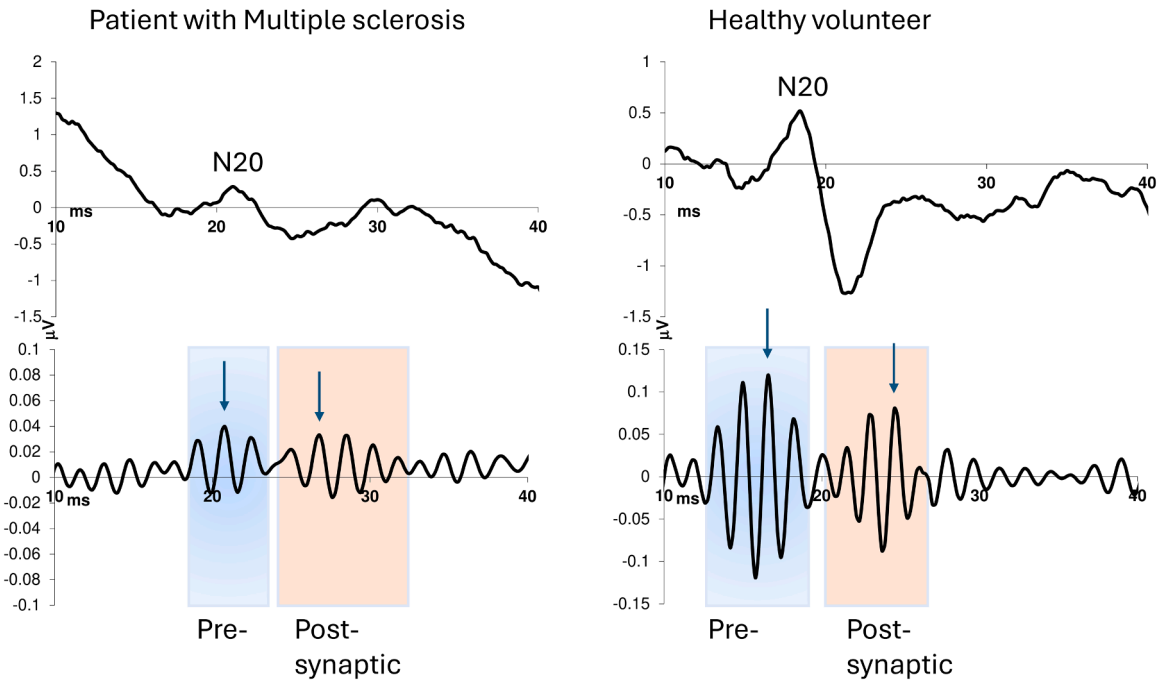

Fig. 2. The upper panel displays broadband SSEP explicative recordings from a patient with multiple sclerosis (MS) and a healthy volunteer exhibiting the N20 peak. The lower panel displays the identical recording after to the application of a 450–750 Hz digital band-pass filter, facilitating the analysis of high-frequency oscillations (HFOs), specifically the pre-synaptic oscillation burst (in light blue), indicative of thalamo-cortical activity, and the post-synaptic burst (in pink), representative of the primary parietal cortex activity. MS patients exhibit prolonged latency and diminished amplitude of the negative oscillatory maximum (shown by the arrow) in both pre- and post-synaptic HFO bursts.

Table 3
Parameters of pre- and post-synaptic somatosensory-evoked high-frequency oscillations (HFOs) bursts in healthy volunteers (HVs) and multiple sclerosis (MS) patients. Data are showed as means ± SD (- / + 95% confidence limits) and inferential statistics, including Cohen's d.

Latency (msec)	Pre-synaptic HFOs			Post-synaptic HFOs		
	HVs	MS	Statistics	HVs	MS	Statistics
	16.9 ± 2.1 (15.7 – 18.2)	19.6 ± 3.4 (18.4 – 20.9)	T = 3.08, p = 0.004, d = −0.96	23.4 ± 2.3 (21.8 – 25.1)	27.4 ± 4.8 (25.8 – 29.1)	U = 97.0, p = 0.003, d = −1.07
Amplitude (μV)	0.048 ± 0.022 (0.040 – 0.056)	0.023 ± 0.012 (0.014 – 0.031)	T = 4.39, p < 0.001, d = 1.40	0.031 ± 0.015 (0.025 – 0.037)	0.020 ± 0.012 (0.014 – 0.027)	T = 2.49, p = 0.017, d = 0.81
Number of Peaks (n)	4.20 ± 0.63 (3.84 – 4.73)	4.50 ± 1.19 (4.05 – 4.95)	T = 0.68, p = 0.498, d = −0.32	3.70 ± 0.67 (3.42 – 4.20)	3.70 ± 0.67 (3.65 – 4.45)	T = 0.87, p = 0.387, d = −0.40
Burst durations (msec)	6.97 ± 1.11 (6.50 – 7.89)	7.93 ± 1.83 (7.22 – 8.64)	T = 1.49, p = 0.145, d = −0.64	6.18 ± 1.19 (5.79 – 7.26)	6.99 ± 1.99 (6.24 – 7.75)	T = 0.89, p = 0.374, d = −0.50
Intra-burst frequency (Hz)	601.40 ± 37.08 (588.49 – 625.07)	585.28 ± 46.63 (566.54 – 604.02)	T = 1.66, p = 0.105, d = 0.38)	599.25 ± 46.96 (603.43 – 648.85	582.64 ± 44.99 (559.37 – 605.92)	T = 2.71, p = 0.01, d = 0.36

inhibition (SAI) (Di Lazzaro et al., 2000; Turco et al., 2018), which point toward a failure of cholinergic and GABAergic circuits in the sensorimotor cortex during exhaustive tasks in MS patients (Brotherton et al., 2024).

Based on these findings in MS pathophysiology, we might postulate an additional GABAergic and cholinergic mechanism responsible for the diminished amplitude and prolonged latency of thalamocortical high-frequency oscillations. We propose that the identical mechanism of diminished GABA availability at the cortex level may elucidate the notable decrease in the intra-burst frequency of the mostly cortical post-synaptic component in our MS patients.

4.2. *Relationship between thalamocortical network activity and fatigue*

The thalamus, serving as a gateway for cortical regions and a relay between cortical and subcortical structures (Minagar et al., 2013), together with its network connectivity to various brain areas, has increasingly attracted interest in the investigation of the pathophysiology of MS-related fatigue (Capone et al., 2020a). Advanced neuroimaging techniques revealed indirect indicators of demyelination and axonal degeneration in the thalamus of fatigued MS patients (Derache et al., 2013; Niepel et al., 2006; Wilting et al., 2016).

Neuroimaging evidence supports the central role of the thalamus in MS-related fatigue, showing that altered resting-state functional connectivity (RS FC) in thalamic subregions correlates significantly with clinical fatigue scores (Hidalgo de la Cruz et al., 2018). While initial cortical-subcortical overactivation may reflect compensation to structural damage, the eventual exhaustion of functional reserve leads to persistent fatigue (Capone et al., 2020a). Such functional decline is likely ultimately underpinned by thalamic microstructural injury and atrophy (Kipp et al., 2015).

In MS patients, it was observed that repeated exertions results in diminished strength performance and a reduction in the total area of HFOs, mostly attributable to a drop in the early component (Capone et al., 2020b). To maintain extended efforts, it is necessary to enhance the recruitment of thalamic neurons; however, atrophy and neuronal injury may impede this process. Furthermore, following multiple attempts, the duration of the late component HFO is selectively extended

Table 4
Means ± SD (- / + 95% confidence limits) of neuro-ophthalmological descriptive features of healthy volunteers (HVs) and multiple sclerosis (MS) patients eyes. BCVA= Best Corrected Visual Acuity; LogMAR= logarithm of the Minimum Angle of Resolution; N=number of eyes; VEP= visual evoked potentials; IT= implicit time; A= amplitude. Statistics includes Cohen's d.

	HVs	MS	Statistics
BCVA (LogMAR)	0.00 ± 0.00	0.00 ± 0.00	
Intraocular pressure (mmHg)	13.22 ± 1.30 (12.76 – 13.71)	12.75 ± 1.70 (12.34 – 13.29)	t = 1.40, p = 0.169, d = 0.31
Refractive error (dioptre spherical equivalent)	0.75 ± 0.25 (0.65 – 0.86)	0.65 ± 0.25 (0.56 – 0.77)	t = 1.81, p = 0.078, d = 0.40
Chromatic perception (Ishihara plates ratio)	1.00 ± 0.00	1.00 ± 0.00	
60′ VEP P100 IT (msec)	102.72 ± 2.15 (101.88 – 103.66)	103.32 ± 2.52 (102.52 – 104.31)	t = 1.15, p = 0.255, d = −0.26
15′ VEP P100 IT (msec)	104.88 ± 2.83 (103.96 – 105.85)	105.81 ± 1.84 (104.80 – 106.69)	t = 1.77, p = 0.085, d = −0.39
60′ VEP N75-P100 (μV)	10.01 ± 1.65 (9.45 – 10.61)	9.81 ± 1.74 (9.10 – 10.27)	t = 0.53, p = 0.599, d = 0.12
15′ VEP N75-P100 (μV)	9.56 ± 1.54 (8.78 – 10.21)	9.46 ± 2.04 (8.67 – 10.10)	t = 0.25, p = 0.805, d = 0.06

(Capone et al., 2020b), potentially attributable to plasticity in GABAergic fibres within the somatosensory cortex, which is essential for compensating grey and white matter damage, yet may also be associated with dysfunction in thalamo-cortical connectivity (Capone et al., 2020a).

In our work, extended latencies of the negative oscillatory peak of pre-synaptic HFO bursts are positively connected with self-reported fatigue. The pre-synaptic burst, indicative of action potentials in the terminal tract of thalamo-cortical axons, suggests the concept that an aberrant thalamo-cortical network may be associated with fatigue in MS. It is noteworthy that fatigue levels did not correlate with SSEP habituation levels. The latter values were consistent between fatigued and healthy individuals. This outcome diverges from earlier findings obtained by VEPs and pattern electroretinography, which indicated an amplitude habituation deficiency (Bir et al., 2013, p. 20; Fadda et al., 2013). Habituation is a fundamental mechanism that mitigates information overload and preserves energy resources inside the central nervous system. The absence of correlation between somatosensory habituation levels and fatigue levels in our patients suggests, firstly, that the neural mechanisms governing these two phenomena are likely distinct, and secondly, that fatigue in MS patients is probably not attributable to excessive depletion of energy reserves.

This study, like many others, possesses limitations. First, the limited sample size of the study, with the lack of formal sample size calculation, may restrict the immediate generalisability of the findings. Second, patients were not stratified for disease-modifying drugs, thus we cannot rule out an effect of therapy on fatigue levels and neurophysiological findings. Another limitation is the lack of neuroimaging data about the integrity and the functionality of the thalamo-cortical pathway in our sample. No specific neuroimaging studies were performed to evaluate the presence of structural and/or functional changes in the thalamus although the recording of normal N20 warranted the integrity of the somatosensory pathway conveying signals from upper limb to cortex. Several factors (such as demographics, comorbidity, depression, pain, sleep problems) can contribute to pathogenesis or exacerbate the manifestations and perception of MS-related fatigue. The present study did not specifically assess these factors, nor were the results controlled for these variables of interest. Additional limitations of this study encompass the FSS's primary focus on the physical aspect of fatigue, the lack of generalisability of the findings to other forms of MS, and the study's cross-sectional design.

5. Conclusions

Our data indicated a decrease and slowing of somatosensory thalamocortical network activity in individuals with MS. We additionally noted that fatigue levels are correlated with thalamocortical activity levels, but not with habituation levels. Given the well-known macro- and micro-structural abnormalities of thalamus identified on MRI in MS patients (Droby et al., 2020; Hidalgo de la Cruz et al., 2018), additional research is required to better explore the correlation between these structural findings and functional somatosensory thalamocortical activity as well as fatigue levels, using neurophysiological tools.

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Declaration of competing interest

The authors declare no competing interests with respect to the

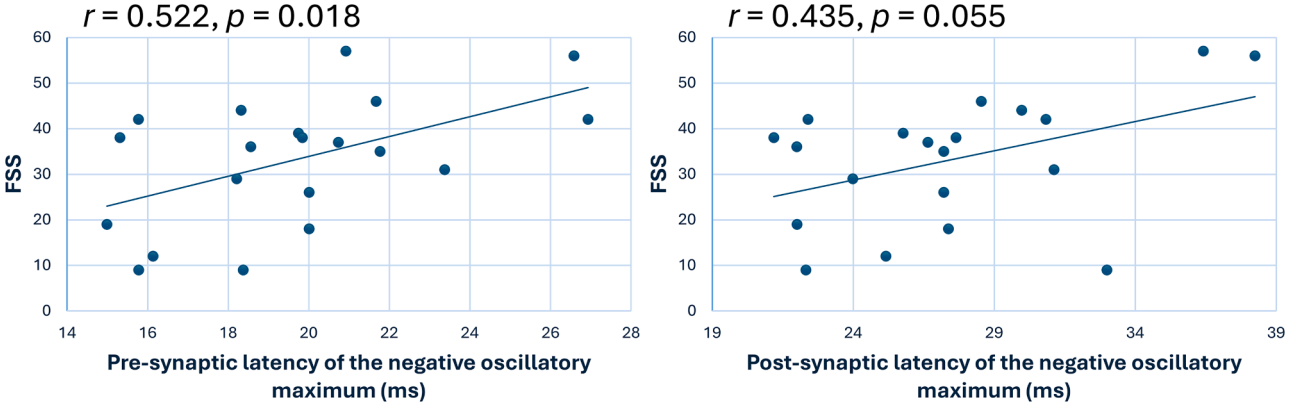

Fig. 3. Correlation between the pre- (right side) and post-synaptic (left side) latency of negative oscillatory maximum (msec) and the fatigue severity scale (FSS) in patients with multiple sclerosis.

research, authorship, and/or publication of this article.

CRediT authorship contribution statement

Benedetta Pitzalis: Investigation. **Ludovica Ciavatta:** Investigation. **Giorgio Leodori:** Writing – review & editing. **Lucia Ziccardi:** Writing – review & editing. **Vincenzo Parisi:** Writing – review & editing. **Antonella Conte:** Writing – review & editing. **Fioravante Capone:** Writing – review & editing. **Vincenzo Di Lazzaro:** Writing – review & editing. **Flavia Pauri:** Writing – original draft. **Gianluca Coppola:** Writing – original draft, Project administration, Formal analysis.

Declaration of competing interest

We declare that we have nothing to disclose related to the present manuscript entitled "Contribution of thalamus-cortex network and cortical habituation mechanisms in multiple sclerosis".

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