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Intracortical GABAergic dysfunction in patients with fatigue and dysexecutive syndrome after COVID-19

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HIGHLIGHTS

- First study applying TMS in patients with fatigue and dysexecutive syndrome in the aftermath of COVID-19.
- TMS studies revealed marked impairment of GABAergic intracortical inhibitory circuits within primary motor cortex.
- TMS may serve as diagnostic tool in cognitive disturbances and fatigue in post-COVID-19 patients.

ABSTRACT

Objective: A high proportion of patients experience fatigue and impairment of cognitive functions after coronavirus disease 2019 (COVID-19). Here we applied transcranial magnetic stimulation (TMS) to explore the activity of the main inhibitory intracortical circuits within the primary motor cortex (M1) in a sample of patients complaining of fatigue and presenting executive dysfunction after resolution of COVID-19 with neurological manifestations.

Methods: Twelve patients who recovered from typical COVID-19 pneumonia with neurological complications and complained of profound physical and mental fatigue underwent, 9 to 13 weeks from disease onset, a psychometric evaluation including a self-reported fatigue numeric-rating scale (FRS, Fatigue Rating Scale) and the Frontal Assessment Battery (FAB). Intracortical activity was evaluated by means of well-established TMS protocols including short-interval intracortical inhibition (SICI), reflecting $GABA_A$-mediated inhibition, long-interval intracortical inhibition (LICI), a marker of $GABA_B$ receptor activity, and short-latency afferent inhibition (SAI) that indexes central cholinergic transmission. TMS data were compared to those obtained in a control group of ten healthy subjects (HS) matched by age, sex and education level.

Results: Post-COVID-19 patients reported marked fatigue according to FRS score (8.1 ± 1.7) and presented pathological scores at the FAB based on Italian normative data (12.2 ± 0.7). TMS revealed marked reduction of SICI, and disruption of LICI as compared to HS. SAI was also slightly diminished.

Abbreviations: TMS, transcranial magnetic stimulation; GABA, gamma aminobutyric acid; SICI, short-interval intracortical inhibition; LICI, long-interval intracortical inhibition; SAI, short-latency afferent inhibition; FRS, Fatigue Rating Scale; FAB, frontal assessment battery.

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Conclusions: The present study documents for the first time reduced GABAergic inhibition in the M1 in patients who recovered from COVID-19 with neurological complications and manifested fatigue and dysexecutive syndrome.
Significance: TMS may serve as diagnostic tool in cognitive disturbances and fatigue in post-COVID-19 patients.

1. Introduction

Patients affected by coronavirus disease 19 (COVID-19) may develop a wide spectrum of neurological manifestations affecting central and peripheral nervous system that have been linked to hyper-inflammatory reaction to "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) infection (Ellul et al., 2020). However, autoimmune pathology following dysregulation of the host immune defence and direct infection of the nervous system via hematogenous or neuronal retrograde routes cannot be excluded (Ellul et al., 2020). In 64 patients with COVID-19-associated neurologic manifestations, 36 presented with abnormal MRI, most frequently ischemic strokes, leptomeningeal enhancement, and encephalitis (Kremer et al., 2020). Eight patients with COVID-19-associated encephalopathy had anti–SARS-CoV-2 antibodies detected in their cerebrospinal fluid (Alexopoulos et al., 2020). Even after the resolution of the acute disease, patients manifest a plethora of long-lasting symptoms. Among them, a high proportion of individuals (up to 53.1%) experience mental and physical fatigue (Carfì et al., 2020; El Sayed et al., 2020; Ferraro et al., 2021; Ortelli et al., 2020; Townsend et al., 2020) and present with dysexecutive syndrome mainly concerning attentive deficits and reduced cognitive control (Helms et al., 2020; Ortelli et al., 2020). Despite this evidence, impact of COVID-19 on cortical activity has so far not been studied. Here we used transcranial magnetic stimulation (TMS) to investigate the functional integrity of intracortical inhibitory circuits within the primary motor cortex (M1) in a sample of patients who recovered from COVID-19 with neurological complications and presented fatigue and dysexecutive syndrome as long-lasting sequelae. Paired-pulse TMS protocols allow indeed exploring inhibitory or excitatory intracortical networks depending on the intensity and interstimulus interval (ISI) used. The role of inhibitory neuronal networks on physiological brain functions has been thoroughly demonstrated (Tremblay et al., 2016).

Maladaptation of cortical processes related to degeneration of inhibitory GABAergic intracortical circuits within the M1 has been reported in various affections of the central nervous system inducing central fatigue. Short-interval intracortical inhibition (SICI) was found to be reduced in patients with multiple sclerosis (Liepert et al., 2005), encephalopathy following primary biliary cirrhosis (McDonald et al., 2010), and amyotrophic lateral sclerosis (Vucic et al., 2011), who experienced profound fatigue. Furthermore, impairment of SICI and of long-interval intracortical inhibition (LICI) was a specific finding in frontotemporal dementia, in which executive dysfunction is a prominent feature (Benussi et al., 2017).

We hypothesized that fatigue and deficit of frontal cognitive functions in post- COVID-19 patients could be underlined by functional impairment of the main inhibitory circuits in the M1 and searched therefore, by means of TMS, for specific alteration of related neurophysiological markers. We applied a well-established paired-pulse TMS protocol to study SICI and intracortical facilitation (ICF) (Kujirai et al., 1993). SICI is thought to represent $GABA_A$-receptor-mediated fast inhibitory post-synaptic potentials (IPSPs) in corticospinal neurons (Ziemann et al., 2015). ICF reflects mainly glutamatergic intracortical excitatory transmission although it is a net facilitation that amalgamates inhibition from the tail of the $GABA_A$-receptor-mediated SICI (Ziemann et al., 2015). We also assessed LICI (Valls-Solé et al., 1992), which is considered to be a phenomenon dependent on slow IPSPs mediated through $GABA_B$-receptors (Ziemann et al., 2015). Finally, we explored short-latency afferent inhibition (SAI), a marker of inhibitory sensorimotor integration that depends mainly on the excitatory effect of cholinergic thalamocortical projections on the inhibitory GABAergic cortical network (Alle et al., 2009).

2. Methods

2.1. Participants

We studied twelve patients (2 female; age 67 ± 9.6 years; 11 right-handers; education level 11.8 ± 3.5 years) who recovered from COVID-19 pneumonia (confirmed by molecular nasopharyngeal swab test and by chest-computer tomography) with disparate neurological complications (critical illness neuropathy and myopathy, Guillain-Barré syndrome, encephalopathy and stroke) at post-acute stage, 9 to 13 weeks after disease onset, at the end of the neurorehabilitation period.

Ten healthy subjects (HS) matched by age, sex, and education level were recruited as control group (3 females; age 61 ± 8.2 years; 10 right-handers; education level 12.8 ± 3.8 years).

During the acute infection, COVID-19 patients sustained bilateral severe pneumonia, prolonged intensive care treatment, and a hyper-inflammatory state, as demonstrated by both markedly elevated C-reactive protein (CRP) and interleukine-6 (IL-6) serum levels.

At the time of the study they had almost recovered from their neurological symptoms but complained of profound fatigue.

Further inclusion criteria were: a) absence of neurological disorders prior to COVID-19, b) absence of prior or current diagnosis of psychiatric, endocrine, metabolic or cardiopulmonary conditions related to fatigue, c) absence of dyspnoea or other long-lasting sequelae of interstitial COVID-19 pneumonia, d) absence of anaemia, e) no treatment with corticosteroids, antihistaminic, antihypertensive, diuretic, or hypnotic drugs at the time of study.

2.2. Psychometric evaluation

Post-COVID-19 patients performed self-evaluation of perceived psycho-physical fatigue during the preceding week by means of a single numeric rating scale, the Fatigue Rating Scale (FRS, 0: no fatigue; 10: extreme fatigue, cut-off for abnormality: 6) (Mordillo-Mateos et al., 2019).

They also underwent evaluation of executive functions by means of the Frontal Assessment Battery (FAB) (Dubois et al., 2000), consisting of six tasks: conceptualization and abstract reasoning through a similarities judgment task; mental flexibility through a phonetic-cue word generation task; motor programming and executive control of action through Luriás series reproduction

task; resistance to interference through a go-no-go task; inhibitory control and self-regulation through a conflicting instruction task, and environmental autonomy through the prehension behavior evaluation. Each item provides a score ranging from 0 to 3, for a total score of eighteen. FAB scores corrected for age and education lower than 13.48 were reported abnormal, based on Italian normative data (Appollonio et al., 2005).

2.3. TMs

During the experiments, the patients were sitting comfortably in an armchair with their eyes open.

First, we recorded motor evoked potentials (MEPs) in the relaxed first dorsal interosseous (FDI) muscle. TMS was delivered over the left primary motor hand area through a tangentially oriented 7 cm figure-of-eight coil connected via a Bistim module with two Magstim 200 stimulators (Magstim Company, Whitland, Dyfed, UK) and placed over the optimal site for eliciting MEPs in the contralateral FDI muscle. The coil position was continuously monitored during the entire experiment.

The resting motor threshold (RMT) was defined as the lowest TMS intensity (expressed in percentage of the maximum stimulator output) that evoked MEPs of at least 50 μV peak-to-peak amplitude in five of ten successive trials (Rossini et al., 2015).

Then we evaluated SICI at 2 and 3 ms interstimulus intervals (ISIs) and ICF at ISI 10 and 15 ms (Kujirai et al., 1993). The stimulation intensity of the conditioning stimulus was set at 70% RMT. The stimulation intensity of the test stimulus was set at 130% RMT and adjusted to elicit stable MEPs of approximately 1 mV peak-to-peak amplitude.

Smaller but stable MEPs were accepted in those patients presenting neuropathy or myopathy (Table 1).

Subsequently, we evaluated LICI at ISI 50 and 100 ms (Valls-Solé et al., 1992). The stimulation intensity both of the conditioning and test stimulus was set at 130% RMT.

Finally, we assessed SAI in order to evaluate motor cortex inhibition induced by sensory afferents. The conditioning stimulus to the ulnar nerve at the wrist (at an intensity just above motor threshold for evoking a visible twitch of the interossei muscles) preceded the TMS by two different ISIs (+0 and + 4 ms, determined relative to the latency of the N20 component of the somatosensory evoked potentials) (Di Lazzaro et al., 2007; Tokimura et al., 2000). The intensity of the TMS test pulse over M1 was adjusted to elicit stable MEPs of approximately 1 mV peak-to-peak amplitude in the relaxed FDI. We recorded somatosensory evoked potentials and measured N20 onset latency as previously described (Cruccu et al., 2008).

In all paradigms, ten stimuli were delivered for each ISI and twenty for the test condition in a pseudo-randomized sequence, considered to be a reasonable number of trials in a population MEP amplitude analysis (Ammann et al., 2020).

Responses were amplified with a Digitimer D440-4 (Digitimer Ltd., Welwyn Garden City, UK), at a sampling rate of 5 kHz, filtered at 20 and 2000 Hz, and fed to a computer using SIGNAL software (CED, Cambridge, UK). For all protocols, the amplitude of the conditioned responses was expressed as a percentage of the corresponding mean unconditioned response.

2.4. Statistics

For SICI-ICF, a two-factor repeated-measures ANOVA was performed with between-subjects factor GROUP (2 levels: patients, HS) and within-subjects factor ISI (4 levels: 2, 3, 10, and 15 ms). For both, LICI and SAI, respective two-factor repeated-measures ANOVAs were performed with between-subjects factor GROUP (2 levels: patients, HS) and within-subjects factor ISI (LICI: 50 and 100 ms; SAI: N20 + 0 and N20 + 4 ms).

Sphericity of data was assessed according to Mauchly, and when violated, Greenhouse–Geisser correction was applied accordingly. Significant main effects were followed-up with unpaired t tests.

We finally performed a correlation analysis with non-parametric Spearman-rho testing to account for the small number of items. We analysed possible relations among the percent change in patients' conditioned MEP size at each ISI of the SICI-ICF, LICI, and SAI tests with individual FRS and FAB scores. A p value < 0.05 was considered significant.

2.5. Standard protocol approvals and patient consents

The study was approved by Human Research Ethics Committee of the Province of Bolzano, Italy (65–2020).

Written informed consent was obtained from all participants, who provided authorization for disclosure of any information that may be published.

3. Results

Patients and controls tolerated the procedures well.

Table 1 summarizes demographic, clinical, laboratory, neuropsychological and neurophysiological data.

All patients reported marked fatigue on the FRS (mean score 8.1 ± 1.7). Moreover they presented diminished executive functions, as documented by abnormal scores corrected for age and education on the FAB (12.2 ± 0.7) (Appollonio et al., 2005).

The results of the various TMS protocols are shown in Table 2 and Fig. 1.

Repeated-measures ANOVA performed on the SICI-ICF data showed a significant main effect of ISI ($F_{1.831,36.619}$ = 22.770; p < 0.001; η_p^2 = 0.532) and a significant GROUP × ISI interaction ($F_{1.831,36.619}$ = 5.615; p = 0.009; η_p^2 = 0.219). Post-hoc analysis showed less SICI at ISI 2 ms (p < 0.001) and 3 ms (p < 0.01) in patients vs. HS (Fig. 1A). For LICI, repeated-measures ANOVA revealed a significant main effect of GROUP ($F_{1,20}$ = 19.075; p < 0.001; η_p^2 = 0.488) but not of ISI, nor an interaction of GROUP × ISI. Post-hoc analysis revealed less LICI at ISI 50 ms (p = 0.008) and 100 ms (p < 0.001) in patients vs. HS (Fig. 1B). For SAI, repeated-measures ANOVA depicted a significant main effect of GROUP ($F_{1,20}$ = 5.612; p = 0.028; η_p^2 = 0.219), but not of ISI, nor an interaction of GROUP × ISI. Post-hoc analysis revealed significantly less SAI at ISI N20 + 0 ms (p = 0.003) in patients vs. HS, while the difference did not reach statistical significance at ISI N20 + 4 ms (Fig. 1C).

The correlation analysis highlighted a negative association (ρ = -0.643, p = 0.024) between conditioned MEP size at ISI N20 + 0 ms in the SAI protocol and the patients' FAB scores.

4. Discussion

The present findings provide neurophysiological evidence of severe impairment of GABA-ergic intracortical circuits in patients who recovered from COVID-19 with various central and peripheral neurological manifestations and who presented fatigue and impairment of executive functions.

Compared to HS, post-COVID-19 patients exhibited reduced inhibition within the M1 as evidenced by disruption of $GABA_A$ mediated SICI, at ISI 2 ms and 3 ms, and of $GABA_B$ mediated LICI, at ISI 50 ms and 100 ms.

ICF, which is thought to largely reflect excitatory glutamatergic transmission through the NMDA receptor, was not affected.

Table 1
Demographic, clinical, laboratory, neurophysiological and neuropsychological data.

Patient	Age [years]	Sex	Diagnosis	Clinical features at admission in neurorehabilitation	Clinical features at the time of TMS study	COVID-19 duration until TMS study [weeks]	Peak IL-6 level [pg/ml] [<7]	Peak CRP level [mg/l] [<0.8]	RMT [% MSO]	AMT [% MSO]	MEP Amplitude (mean of 5 trials) [mV]	FRS [0–10]	FAB [0–18]
1	65	M	CINM	Moderate flaccid tetraparesis, areflexia; deep sensory disturbances in lower limbs	Fatigue; dysexecutive syndrome	11	401	18.7	42	39	1.4	7	12.4
2	60	M	CINM	Flaccid tetraparesis,muscle atrophy, areflexia	Mild distal paresis MRC 4/5; fatigue; dysexecutive syndrome	10	555	15.9	50	46	0.4	10	12.5
3	62	M	CIN	Predominantly distal tetraparesis, hyporeflexia; anosmia	Fatigue; dysexecutive syndrome; anosmia	11	225	17.1	39	35	0.9	10	13.0
4	71	M	Encephalopathy	Severe cognitive impairment; dysphagia; anosmia	Severe multidomain cognitive impairment with predominant dysexecutive syndrome; fatigue; anosmia	9	635	25.2	40	38	0.6	6	10.9
5	79	M	GBS (AIDP); mild cognitive impairment	Predominantly distal tetraparesis, areflexia; mild superficial and deep sensory disturbances; deficit in attentional processes and impulse control; anosmia	Severe dysexecutive syndrome; fatigue; anosmia	12	214	39.3	38	35	1.9	9	11.5
6	75	F	Stroke (rMCA)	Mild left hemiparesis with hemisensory loss; left hemispatial neglect	Mild distal paresis in left upper limb (MRC 4/5); dysexecutive syndrome; fatigue.	12	N/A	22.4	47	44	0.4	6	11.7
7	48	M	Myopathy	Limb-girdle muscle atrophy and paresis; mild myalgia	Mild proximal paresis (MRC 4/5); dysexecutive syndrome; fatigue.	13	6386	20.1	50	46	0.5	6	12.9
8	56	M	Myopathy	Limb-girdle muscle atrophy and paresis; myalgia; anosmia, dysgeusia	Dysexecutive syndrome; fatigue.	13	2418	34.2	40	37	0.6	10	13.1
9	70	M	GBS (AMAN)	Predominantly distal tetraparesis, areflexia	Mild distal paresis MRC 4/5; fatigue; dysexecutive syndrome	10	688	18.9	52	49	0.5	8	12.4
10	61	F	Encephalopathy	Behavioural changes; primary insomnia, fatigue; anosmia	Dysexecutive syndrome; fatigue.	12	271	25.7	55	48	2.0	10	11.9
11	77	M	Myopathy	Limb-girdle muscle atrophy and paresis; myalgia	Mild proximal paresis (MRC 4/5); dysexecutive syndrome; fatigue.	13	1251	30.4	41	38	0.4	9	12.9
12	80	M	Encephalopathy	Severe cognitive impairment; anosmia	Severe multidomain cognitive impairment with predominant dysexecutive syndrome; fatigue; anosmia	12	129	23.0	42	38	1.3	6	11.5

TMS, transcranial magnetic stimulation; CRP, c-reactive protein; IL-6, interleukin 6; RMT, resting motor threshold; AMT, active motor threshold; MEP, motor evoked potential; MSO, maximum stimulator output; CINM, critical illness neuropathy and myopathy; CIN, critical illness neuropathy; GBS, Guillain-Barré syndrome; AIDP, acute inflammatory demyelinating polyneuropathy; AMAN, acute motor axonal neuropathy; rMCA, right middle cerebral artery; FRS, fatigue rating scale; FAB, frontal assessment battery, scores corrected for age and education lower than 13.48 are abnormal, based on Italian normative data (Appollonio et al., 2005).

Table 2
Mean percentage of conditioned divided by unconditioned MEP amplitude (standard error in brackets) in TMS protocols testing short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), long-interval intracortical inhibition (LICI), and short-latency afferent inhibition (SAI) at specified interstimulus intervals (ISI).

		Patients	Controls
SICI	ISI 2 ms	82.1 (6.3)	33.6 (6.1)
	ISI 3 ms	93.5 (11.3)	52.1 (8.3)
ICF	ISI 10 ms	127.6 (16.3)	117.3 (13.3)
	ISI 15 ms	123.5 (11.6)	163.2 (30.5)
LICI	ISI 50 ms	78.6 (10.9)	33.9 (10.3)
	ISI 100 ms	75.6 (8.6)	26.3 (5.6)
SAI	ISI N20 + 0 ms	70.8 (7.9)	42.0 (4.2)
	ISI N20 + 4 ms	69.8 (10.2)	47.8 (7.4)

MEP: motor evoked potential; TMS: transcranial magnetic stimulation.

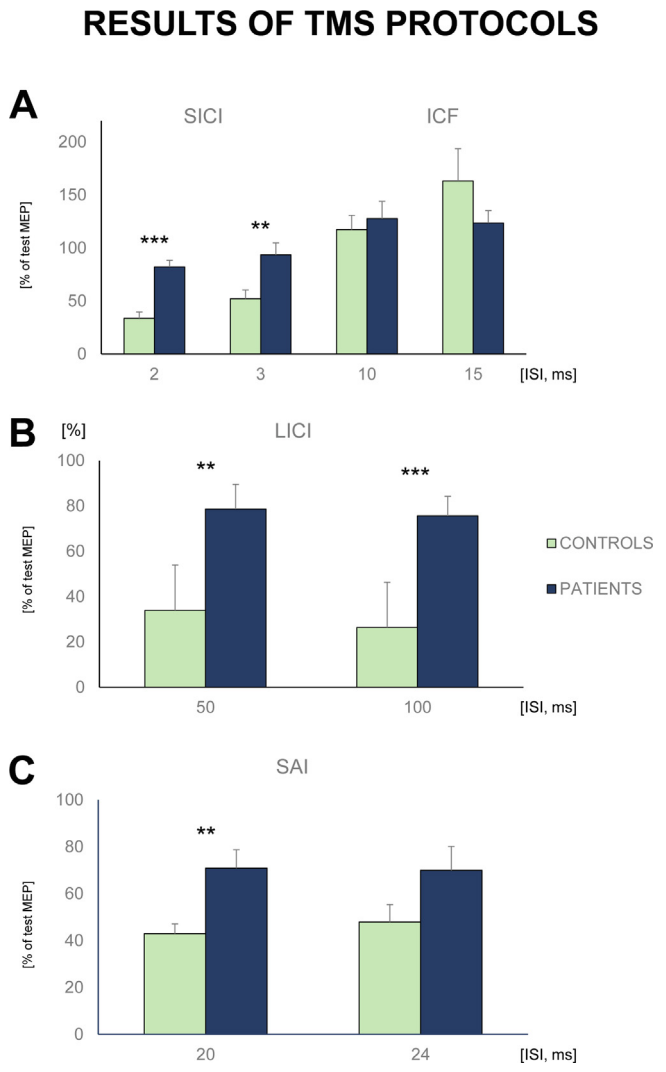

Fig. 1. Results of TMS-protocols. Cortical inhibition tested with different transcranial magnetic stimulation (TMS) protocols in post-COVID-19 patients and in healthy controls. (A) Short-interval intracortical inhibition and facilitation (SICI-ICF) at interstimulus intervals (ISI) 2, 3, 10, and 15 ms; (B) long-interval intracortical inhibition (LICI) at ISIs 50 and 100 ms; (C) short-latency afferent inhibition (SAI) at ISIs N20 + 0 ms and N20 + 4 ms. The columns represent the amplitude of conditioned motor evoked potentials (MEPs) expressed as percentage of the corresponding mean unconditioned response. Whiskers represent standard error. ** = $p < 0.01$, *** = $p < 0.001$.

GABA is the principal inhibitory neurotransmitter in the human nervous system and plays a fundamental role in nearly all neuronal coding and processing throughout the brain. SICI and LICI are impaired in patients with frontotemporal dementia, presenting executive dysfunction (Benussi et al., 2017). Different cognitive abilities, mainly executive functions, are sensitive to cerebral GABA concentrations in the frontal cortex (Sumner et al., 2010; Porges et al., 2017). Impaired GABA-ergic cortical activity could underlie the dysexecutive syndrome common to all patients presented here, regardless of the type of initial neurological complication.

SICI was reported to be reduced in central nervous system disorders inducing chronic fatigue (Liepert et al., 2005; McDonald et al., 2010; Vucic et al., 2011). Interestingly, the same post-COVID-19 patients presented in this study showed, after a fatiguing pinching task, lack of post-exercise depression of MEPs and abnormal prolongation of cortical silent period duration (Ortelli et al., 2020). These findings may reflect the impairment of inhibitory circuits within M1 demonstrated here, with a subsequent alteration of post-exhaustion inhibition of corticomotor excitability.

Neuroinflammation is common to a broad spectrum of neurological disorders (Brambilla, 2019) and may affect GABAergic transmission in neurological disorders (Heneka et al., 2015). The reported patients showed during the acute phase of COVID-19 markedly increased CRP and IL-6 serum levels (Table 1), reliable markers of systemic inflammation. Peripheral cytokines can enter the brain and activate the microglia and the astrocytes inducing neural cytokines release and resulting in brain inflammation (Harry and Kraft, 2008). COVID-19-associated neuroinflammation could be the underpinning of the observed alteration in M1 circuits. On the other hand, prolonged cerebral hypoxia due to SARS-CoV-2-associated pulmonary pathology may also have contributed to the observed phenomenon. Notably, SICI was reduced in patients with chronic obstructive pulmonary disease (Oliviero et al., 2002).

Compared to HS, SAI mechanisms were also significantly reduced in post-COVID-19 patients at ISI N20 + 0 ms, with a similar, but non-significant reduction at N20 + 4 ms. SAI evaluates motor cortex inhibition induced by sensory afferents through inhibitory connections from the primary somatosensory cortex to M1. Both, ISI N20 + 0 ms (Tokimura et al., 2000) and N20 + 4 ms (Di Lazzaro et al., 2007) were previously demonstrated to be effective for strong SAI induction. Reduced excitatory cholinergic projections to M1 GABAergic interneurons could account in part for the observed reduction of GABA networks activity, since SAI is considered to act under the control of SICI circuit (Alle et al., 2009). As a relevant co-finding, there was a negative association between conditioned MEP size at ISI N20 + 0 ms and FAB score (the smaller the conditioned MEPs, i.e., the more efficient SAI, the better the cognitive performance).

SAI reduction was previously found during repetitive non-fatiguing movements inducing MEP depression (Miyaguchi et al., 2017). Furthermore, abnormal SAI findings concurring with central cholinergic dysfunction have been related to dementia (Nardone et al., 2011) and to olfactory dysfunction in idiopathic Parkinson's disease (Versace et al., 2017).

Taken together, the present findings point towards a general reduction of cortical GABAergic and - to a lesser extent - cholinergic activity in post-COVID-19 patients. This alteration could underlie both the reduced cognition and the abnormal fatigue perception and could represent one of the possible mechanisms of COVID-19-related neurotoxicity. TMS may therefore represent a useful diagnostic tool in post-COVID-19 patients suffering from fatigue or cognitive disturbances.

Despite limitations – e.g., the small sample size with sequelae of inhomogeneous neurological affections and the lack of direct detection of inflammatory markers in the patients' cerebrospinal fluid - the present study documents for the first time reduced

GABAergic activity in M1 in patients who recovered from COVID-19 with associated neurological complications and presented with long-lasting fatigue and executive dysfunction.

Further studies need to confirm these neurophysiological abnormalities, to better define their relationship with clinical features and to follow-up patients over time.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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