COGNITION IN MULTIPLE SCLEROSIS

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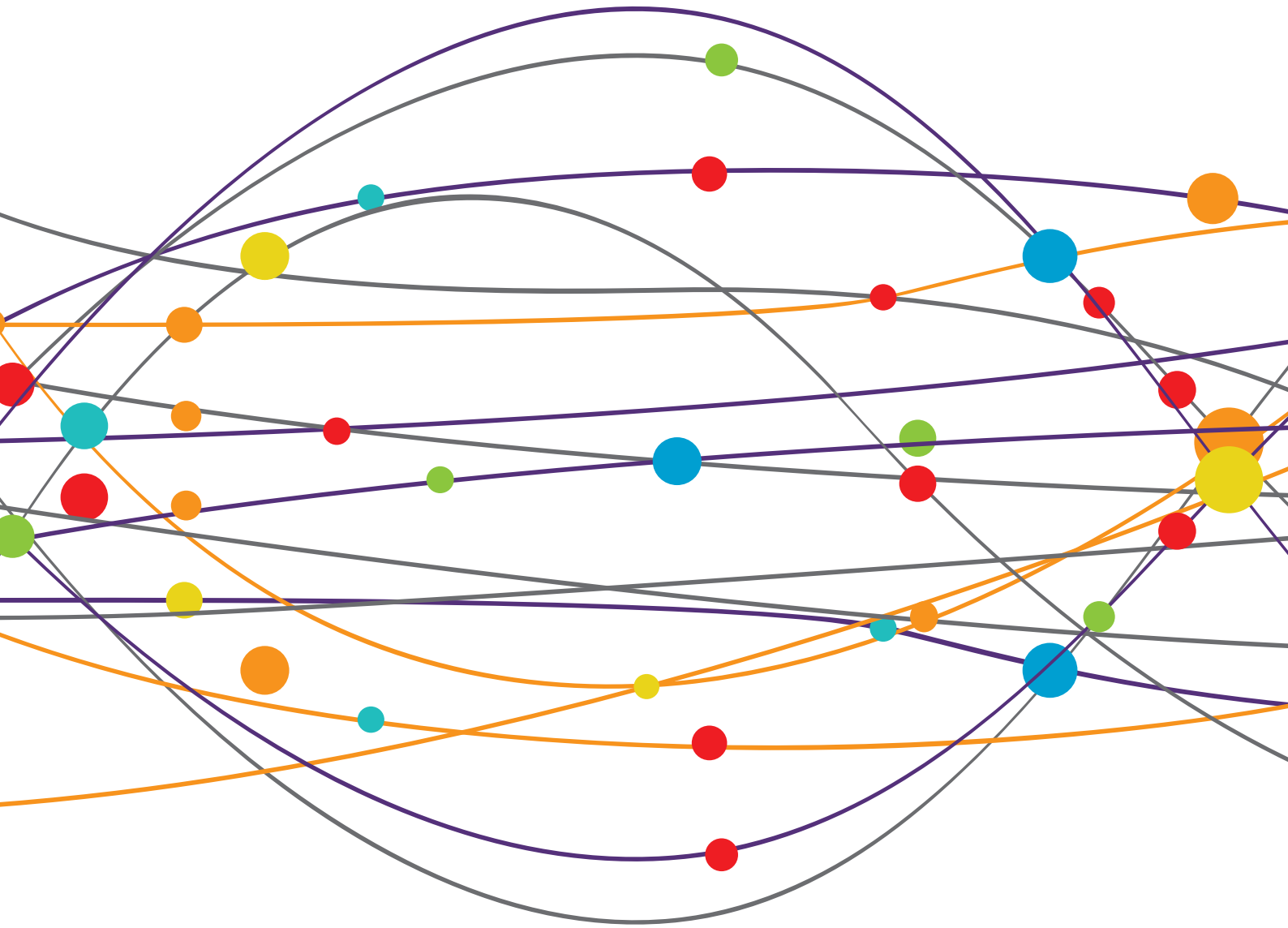

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COGNITION IN MULTIPLE SCLEROSIS

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EDITORIAL
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Editorial: Cognition in Multiple Sclerosis

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Editorial on the Research Topic

Cognition in Multiple Sclerosis

Cognitive impairment (CI) is common in patients with multiple sclerosis (MS), with a prevalence ranging from 34 to 65%, depending on several factors, such as disease duration and age at disease onset. Moreover, accumulating evidence reports that in MS less explored cognitive domains (i.e., theory of mind, pragmatics, meta-cognition, prospective memory) might be also affected in the absence of an overall CI (1). Recently, diverse computerized neuropsychological testing tools have been developed to allow the application of more comprehensive assessment batteries. Yet, understanding of their use remains limited due to the lack of validation studies on larger samples. To date, the pathological brain changes associated with cognitive disability in MS are not fully understood (2). The application of novel advanced imaging techniques has the potential to reveal the mechanisms underpinning both the overall CI and the impairment in selected cognitive domains. Finally, efficient approaches for treating CI are still lacking. Despite the efficacy of disease modifying treatment in preventing cognitive decline, results of clinical trials were disappointing (1).

This Research Topic on CI in MS aims to review studies on this subject. Authors have contributed with 15 works on different aspects of MS-related CI, including works exploring (i) CI assessment tools development and the cognitive processes underlying failure at neuropsychological tests in MS; (ii) correlations between CI and disease biomarkers, (iii) MRI pathological substrates underpinning CI; and (iv) possible therapeutic strategies for CI in MS.

In particular, one main field investigated by the authors relates to self-assessment and perception of cognitive functioning. Riccardi et al. developed a new questionnaire called Sclerosis Multipla Autovalutazione Cognitiva (SMAC), which showed a promising Patient-Reported Outcome to be included in MS neuropsychological evaluation. Another way to explore the experience of individuals living with MS and their cognitive involvement is the Cognitive Assessment Interview (CAI), a patient and informant-based semi-structured interview, which Eilam-Stock et al. demonstrated to add information to both self-report measures and neuropsychological assessment, and to characterize the experience of CI in persons living with MS. Interestingly, a common finding of these works is that caregiver perception is more strongly correlated to the objective cognitive performance of people with MS than a patient's self-judgment. It is worth noting, however, that local norms should be followed when interpreting the results of cognitive tests and the performance of regression-based norms developed in other populations need to be considered when applying them to local populations, even when they are from the same country, as demonstrated by Marrie et al..

The main imaging marker of neurodegeneration in MS is supposed to be brain atrophy, which is associated with cognitive impairment and retinal nerve fiber layer (RNFL) atrophy. Fenu et al. confirmed the role of brain atrophy as a biomarker of CI and highlighted the importance of a

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caregiver's perception for cognitive assessment of patients with MS. In a 5-year follow-up study, Giedraitiene et al. showed that RNFL atrophy and other inflammatory markers, like oligoclonal bands in cerebrospinal fluid were related to cognitive decline in MS patients. Additionally, Portaccio et al. found that Brain Derived Neurotrophic Factor (BDNF) Val66Met polymorphism may have a protective role against cognitive impairment in MS patients.

Diving deeper into the different cognitive functions involved in MS has become a predominant issue. For example, verbal fluency (VF) has been associated with several cognitive functions, but the cognitive processes underlying deficits in this cognitive domain in MS are controversial. Delgado-Álvarez et al. evaluated the cognitive processes related to VF and developed machine-learning algorithms to predict those patients with cognitive deficits using only VF-derived scores. In their work, VF was influenced by many other cognitive processes, mainly including attention-executive functioning, episodic memory, and language. Semantic fluency and clustering were more explained by memory function, while phonemic fluency and switching were more related to executive functioning, supporting that the multiple cognitive components underlying VF tasks in MS could even serve for screening purposes and the detection of executive dysfunction.

Impaired temporal processing of simultaneity/successiveness has been frequently reported in MS, while interval timing has not been investigated in adults nor pediatric MS patients. In pediatric MS patients, Troche et al. suggest that subcortical deficits might underlie typical alterations in speech and visuomotor coordination. However, future studies are needed to confirm these findings.

This special issue also reviews recent advances on MRI techniques and their potential to provide a deeper understanding of the pathological substrates of CI in MS. Zhang et al. summarized recent works assessing the structural and functional connectivity substrates of cognitive impairment in MS, using different diffusion tensor imaging (DTI) measures (e.g., fractional anisotropy, diffusivities) along with tractography-derived white matter (WM), while Amin and Ontaneda review the role of the thalamus in MS. They suggested that thalamic atrophy may represent an ideal biomarker for studies aiming to test neuroprotective strategies or restorative therapies for cognition.

Boscheron et al. explored different patterns of structural and functional connectivity between the hippocampus and the rest of the brain and their possible relevance to memory performances in early MS. They found a differential impairment in memory performance in the early stages of MS and an important interplay between hippocampal-related structural and functional networks and those performances.

Cognitive reserve (CR) could attenuate the impact of the brain burden on cognition in people with MS. Lopez-Soley et al. found that CR has a significant effect on brain structural connectivity in MS patients with severe clinical impairment and it may protect them from cognitive decline regardless of their cognitive status, with brain damage and aging also influencing cognitive performance.

Further knowledge is required regarding the treatment of CI. Hsu et al. studied the effects of transcranial direct current stimulation on cognition, mood, pain, and fatigue in MS through a systematic review and meta-analysis that provided preliminary evidence that transcranial direct current stimulation has a favorable effect on cognitive processing speed, mood disturbance, pain, and fatigue in MS. However, the effects on cognition and fatigue varied based on the specific assessment used.

Interestingly, exercise training was shown to have high potential to beneficially impact cognitive performance in patients with MS. Rademacher et al. demonstrated that high intensity interval training has potentially higher effects on physical fitness and cognition compared to moderate continuous exercise, with a larger beneficial effect in MS patients with impaired cognition than in those with intact cognition. A future randomized controlled trial with cognitive performance as the primary endpoint may confirm the beneficial role of exercise training.

In conclusion, this Research Topic has shown advances in understanding the pathogenic substrates of CI in MS and suggests promising strategies to assess the involvement of different cognitive domains. These findings could contribute to improving the personalized care of CI in people with MS.

AUTHOR CONTRIBUTIONS

RC and RL: literature search and drafting the manuscript. AC and MDF: literature search and revising the manuscript. All authors contributed to the article and approved the submitted version.

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PERSPECTIVE
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Why Cognitive–Cognitive Dual-Task Testing Assessment Should Be Implemented in Studies on Multiple Sclerosis and in Regular Clinical Practice

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Cognitive impairment is prevalent and disabling in multiple sclerosis (MS) and is severely impacting quality of life (QoL). Aside its routine assessment in clinical care, it should more often be implemented as endpoint/outcome measure in clinical trials. However, a fundamental aspect—often neglected in clinical practice and clinical trials—is the assessment of multi-tasking and dual-tasking abilities. In this perspective article, we outline why, given the nature of MS, particularly the assessment of "cognitive–cognitive dual-tasking" is relevant in MS. We delineate how knowledge from basic cognitive science can inform the assessment of this important cognitive impairment in MS. Finally, we outline how the assessment of "cognitive–cognitive dual-tasking" can be implemented in computer-based screening tools (e-health devices) that can be used not only in clinical diagnostics but also in clinical trials.

Keywords: multiple sclerosis, cognition, dual tasking, e-health, neuropsychology

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INTRODUCTION

Cognitive impairment is prevalent at all phases and all subtypes of multiple sclerosis (MS). It remains one of the major causes of neurological disability in young and middle-aged adults suffering from the disease (1). The severity and the type of cognitive impairment vary considerably among individuals and can be observed both in early and in later stages. The usual neurological examination fails to detect emerging cognitive deficits; self-reported cognitive complaints by the patients can be confounded by other subjective symptoms (2), so the assessment of cognitive functions should become a cornerstone in routine clinical care of MS patients and is also increasingly considered as an important endpoint in clinical trials (3). Especially with regard to the inclusion of cognitive tests in clinical trials, it is essential that the tests are reliable and quickly feasible. Based on these grounds, especially the symbol digit modalities tests (SDMT) has been included in recent clinical trials. This is also reasonable because the SDMT has been considered to reflect a reliable and relevant cognitive screening instrument in MS (4, 5). The SDMT mainly measures perceptual and attentional speed. Although these are central dysfunctions in MS and, of course, relevant for the patients, MS patients also complain about difficulties when being confronted with "multi-tasking" situations (e.g., in job occupation) (1). Although deficits in

these abilities are frequently reported by MS patients, they are not routinely examined, which is a fundamental shortcoming (6). Often there is a strong discrepancy in a patient's statements about difficulties occurring in daily life and the pattern of the neuropsychological profile as revealed by routinely applied neuropsychological test (batteries) in MS. This is likely the case because current testings (including the SDMT) fall short of examining relevant cognitive dual- or multi-tasking abilities. Distinctions have been made between different forms of multi-tasking (6), and purely cognitive dual-tasking situations have been distinguished from situations in which cognitive and motor demands are imposed in parallel—that is, a distinction between cognitive–cognitive and cognitive–motor dual-tasking situation has been made. The latter (cognitive–motor dual-tasking situations) has already been subject to intense research in MS, and several studies and review articles have been published on walking and postural balance (7–10). However, these sorts of dual-tasking assessment require specialized hard and software packages and cumbersome presentation devices. The clinical usage and the dissemination of "dual-tasking assessments" are strongly facilitated, and their acceptance is increased if a test is short and can, ideally, be delivered flexibly (i.e., without specific software requirements and hardware devices in various settings). This is the case for cognitive–cognitive dual-tasking assessments as outlined below.

COGNITIVE–COGNITIVE DUAL TASKING ASSESSMENT IN THE CONTEXT OF MS

For these matters, especially the assessment of executive functions is central because executive functions predict performance in many daily life relevant areas as [e.g., job occupation (11)]. Especially in MS, this is central since this disease mostly affects people between 20 and 50 years of age. However, executive functions cover a wide range of cognitive processes. Therefore, the exact examination of executive functions often requires various tests so that the examination is time-consuming and rarely feasible when testing novel pharmacological compounds in clinical study settings. A further problem is that many everyday situations do not only claim a circumscribed executive function but represent a mixture of different processes. For this reason, cognitive testing using common neuropsychological tests often falls short (1, 6). Most day-to-day requirements demand several aspects of executive functions simultaneously or in rapid succession.

Dual-tasking, and its assessment, captures the interaction of different executive functions and therefore comes closer to requirements in everyday life. As such, the assessment of dual-tasking functions is important and has an ecological validity for the assessment of cognitive dysfunctions associated with MS (12). Over the last decades, especially research in cognitive and experimental psychology has uncovered the cognitive mechanisms involved in dual- and multi-tasking (13). Importantly, this research has developed rigorous methods (i.e., tests) to assess these functions. One of the most established tests is the psychological refractory period (PRP) task (14) and derivatives of it, like a stop-change task (15). Briefly, people are required to execute two responses in close succession to two different streams of stimuli (e.g., visual and auditory stimuli) (see **Figure 1**, left side, for illustration).

When these responses are demanded in close succession, response selection capacities become overstrained and response selection processes are slowed down (13, 16, 17). Several lines of evidence suggest that these capacities depend on brain structures in the frontal, fronto-central, and parietal regions and are thus organized as long-distance functional neuroanatomical networks (18–27). This is of particular relevance for MS because MS can be seen as a white matter disconnection syndrome (28). Consequently, it has been shown that the ability to select appropriate responses in close succession is predicted by concentrations of serum neurofilament light chain (sNfl) (29). This is of high relevance because there are strong links between the sNfl and the integrity of the white matter structure (30–34), particularly in MS (35, 36). It has been shown (37) that MS patients performed considerably worse than healthy control participants and that the deficits shown by the patients are very likely not due to simple motor deficits.

Aside these neuroanatomical and neurobiological considerations suggesting that the assessment of dual-tasking is relevant in MS, it is important to note that these abilities show little to no susceptibility to learning effects (38). Only after an extensive several-hours training will slight changes in dual-tasking abilities have been documented (39). This is important because the cognitive function (construct) being tested remains reliably testable across different testings (i.e., longitudinally). This contrasts with other tests routinely used to assess cognitive functions in MS, like the PASAT, where strong learning effects are evident and patients report that they do not attend to the task because they already know what is being presented one after another in this task (40). Thus, the assessment of dual-tasking in MS is desirable because the same cognitive function is always tested and not the mixing of learning skills/effects and the cognitive function to be measured. This is furthermore the case because dual-tasking tests (like the PRP) require the responses to be simple visual digits/letters and tones, making it possible to create parallel versions of the task easily and quickly without changing the task difficulty or other characteristics of the test. Data examining the PRP in MS have shown that variations in motor speed (e.g., due to MS-related motor disturbances) do not represent a confound in this task because mostly the accuracy to respond seems to be modulated in MS (37). Moreover, a PRP task can also be applied using voice responses (41). As mentioned, the mechanisms underlying dual-tasking have been subject to intense research for many decades. This has led to an in-depth knowledge of the cognitive subprocesses underlying dual-tasking abilities, with the result that performance in dual-tasking can be described with well-established mathematical models (13). Aside the fact that this underlines the high reliability and validity of the testing procedure, it ensures that the tested cognitive processes are consistent and quantitative. Due to its mathematical modelability, it can be described very clearly under which specific test constellations (test difficulties) differences between persons can be reliably measured. This is important given the (partially) progressive nature of MS and the necessity to be able to track disease progression also at a cognitive level. The strong

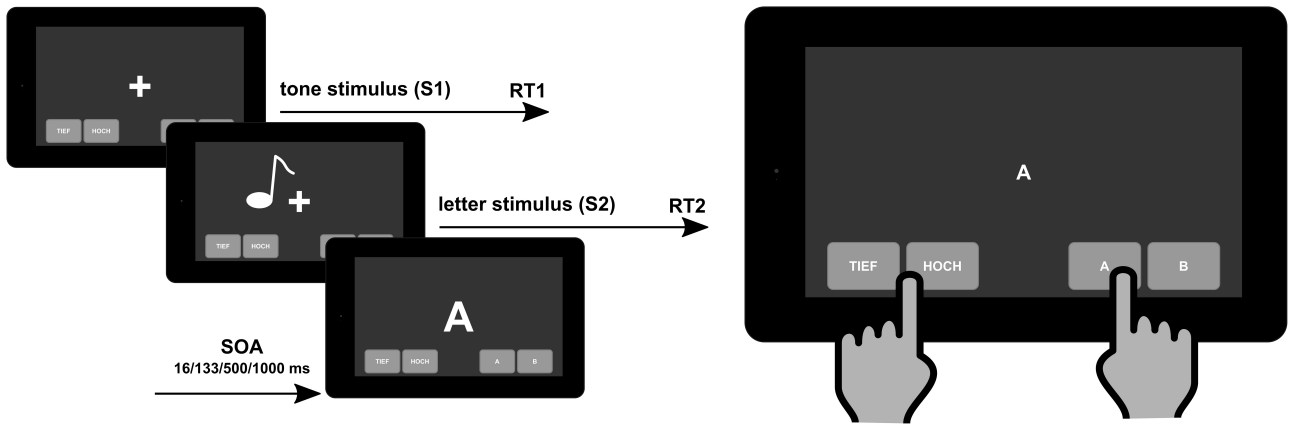

FIGURE 1 | Schematic illustration of the psychological refractory period task to measure "cognitive–cognitive" dual tasking (left) that can be implemented on a mobile e-health device (right). SOA, stimulus onset asynchrony.

conceptual rationale has driven knowledge gain on the cognitive processes being important during dual- and multitasking as well as a task design which ensures that "adaptive" testing is possible and ensures to record longitudinal data with one test without having to change the evaluation instrument. On the same grounds, dual-tasking (and especially the PRP) is reliable and quick to apply, with a high degree of standardization. We have developed a tablet-based solution which can be applied to the patient without extensive explanations. This has two important consequences: first, the test is easy to apply, without intense training of nurses in the clinical real world as well as in study settings and, second and more important, these features of dual-tasking assessment using the PRP enable an assessment using digital health devices which could be applied in MS centers or by the patient himself. This ensures that a dual-tasking assessment using the PRP (and related tasks) is quickly scalable to high case numbers in the context of clinical study situations. In addition, this clinically very relevant test could be transferred to everyday clinical practice to monitor cognitive function longitudinally. The validity of such cognitive tests can be related to two general concepts. The first is construct or concept validity which is quite clear about the dual-task challenge. The other principle includes quantitative interpretability (42). The FDA guidance does not see the treatment benefit as a purely statistical issue but, rather, that it is important to also be able to interpret the observed treatment effect as clinically meaningful. The identification of a score difference can be interpreted as a treatment benefit (i.e., clinically meaningful). Up to now, the SDMT as single, mental processing speed test has been used in clinical studies so far, and it will be important to be able to replicate the results in the domain of executive function which is often defective in MS patients and has the above-mentioned advantages of testing. Data from cognitive tests such as the dual-task test with both statistically and clinically meaningful approaches are needed.

Importantly, the nature and the structure of the PRP dual-task assessment makes it possible to implement this neuropsychological tool in e-health devices [i.e., tablet-based applications that can be on the "bedside" and in routine clinical care in outpatient units (see **Figure 1**, right side)]. The e-health diagnostic tools are helpful instruments to close the supply shortfall in the healthcare system and to improve the care of chronically ill patients because they can present the course of the illness more comprehensively and more accurately than only through standard clinical visits. The MS patients are a suitable group of e-health users (43). Using digital tools, data collection does not increase so much the burden on providers or generate a significant incremental cost, so the proliferation of computerized neuropsychological assessment devices for screening and monitoring cognitive impairment is increasing exponentially (44). In our approach, the digital dual-task assessment tool is implemented in our Multiple Sclerosis Documentation Software MSDS3D and the linked Integrated Care Portal Multiple Sclerosis (IBMS) which contains clinical pathways in a manner which is comprehensible for the patients (45). This is in line with our overall strategy toward personalized MS management such that, in addition to advanced immunological, genetic, and MRI profiling of the individual patient, the clinical profiling of MS patients' inclusive cognition needs to be widely implemented in clinical practice using digital approaches (46).

CONCLUSION

We hope that the self-explanatory reviewed cognitive–cognitive dual-task test will lower the threshold for regular cognitive testing. This has to be proven in future clinical studies. The unsupervised assessment of dual-task function is time-efficient and comes with an advantage that scores could be automatically calculated and sent to the treating neurologist immediately, so regular digital dual-task testing as cognitive monitoring in MS patients will be possible. Ultimately, performing a dual-task test will provide clinicians with an indication of the cognitive performance of patients with MS without the need of a test leader. Follow-up measurement

will be easier to implement and could lead to the timely identification of cognitive decline in patients with MS and subsequently allow for adequate counseling. Focusing at clinical studies, it will be easier to investigate cognitive function as a primary outcome.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All authors conceived the theoretical outline of the article, written, and approved the manuscript.

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Interval Timing in Pediatric Multiple Sclerosis: Impaired in the Subsecond Range but Unimpaired in the One-Second Range

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Background: For adult multiple sclerosis (MS) patients, impaired temporal processing of simultaneity/successiveness has been frequently reported although interval timing has been investigated in neither adult nor pediatric MS patients. We aim to extend previous research in two ways. First, we focus on interval timing (instead of simultaneity/successiveness) and differentiate between sensory-automatic processing of intervals in the subsecond range and cognitive processing of intervals in the one-second range. Second, we investigate whether impaired temporal information processing would also be observable in *pediatric* MS patients' interval timing in the subsecond and one-second ranges.

Methods: Participants were 22 pediatric MS patients and 22 healthy controls, matched for age, gender, and psychometric intelligence as measured by the Culture Fair Test 20-R. They completed two auditory interval-timing tasks with stimuli in the subsecond and one-second ranges, respectively, as well as a frequency discrimination task.

Results: Pediatric MS patients showed impaired interval timing in the subsecond range compared to healthy controls with a mean difference of the difference limen (DL) of 6.3 ms, 95% CI [1.7, 10.9 ms] and an effect size of Cohen's $d = 0.830$. The two groups did not differ significantly in interval timing in the one-second range (mean difference of the DL = 26.9 ms, 95% CI [−14.2, 67.9 ms], Cohen's $d = 0.399$) or in frequency discrimination (mean difference of the DL = 0.4 Hz, 95% CI [−1.1, 1.9 Hz], Cohen's $d = 0.158$).

Conclusion: The results indicate that, in particular, the sensory-automatic processing of intervals in the subsecond range but not the cognitive processing of longer intervals is impaired in pediatric MS patients. This differential pattern of results is unlikely to be

explained by general deficits of auditory information processing. A tentative explanation, to be tested in future studies, points to subcortical deficits in pediatric MS patients, which might also underlie deficits in speech and visuomotor coordination typically reported in pediatric MS patients.

Keywords: cognitive impairment, interval timing, pediatric multiple sclerosis (MS), neuropsychology, distinct timing hypothesis, temporal information processing

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory neurological disease, which leads to demyelination and neuroaxonal injury of the central nervous system and, subsequently, to physical and cognitive impairments. In about 5% of MS patients, onset of the disease is before the age of 18 years (1), and age of onset plays a crucial role in individual differences in neurological and cognitive effects of MS (2). According to Charvet et al. (3), one third of pediatric MS patients suffer from cognitive impairment already in the early phase of the disease. In line with this observation, children and adolescents with MS suffer from substantial brain volume loss already at the time of the first event (4). Pediatric and adult MS patients seem to differ in their cognitive deficits (5), and a longitudinal cohort study demonstrates a more pronounced decline of information-processing efficiency for individuals with pediatric- than adult-onset MS, primarily at the age of about 30 (6). Probably due to the low prevalence of pediatric MS, the manifoldness of cognitive impairments is less well-investigated in pediatric compared to adult MS patients. For example, processing of temporal information has been reported to be impaired in adult MS patients but, to the best of our knowledge, has not been investigated in pediatric MS patients yet.

Temporal information processing does not represent a unitary concept but rather consists of distinct elementary temporal experiences [e.g., (7, 8)]. Researchers interested in the functional relationship between temporal information processing and brain functioning have devoted particular attention to two elementary time experiences: (1) simultaneity and successiveness and (2) interval timing.

Investigations into *simultaneity* and *successiveness* are concerned with the size of the temporal interval between two or more events that is required for them to be perceived as separate events (successiveness) rather than fused as one event (simultaneity). Visual or auditory fusion thresholds, for example, represent an indicator of this type of temporal resolution power for central sensory information processing (9). Over the past six decades, a large number of clinical studies provide convincing evidence for MS patients' significantly impaired visual-temporal resolution ability as indicated by higher fusion thresholds compared to healthy controls (10–14). Although no auditory fusion studies in MS patients seem to exist, important clues for impaired auditory temporal resolution ability comes from a recent study by Valadbeigi et al. (15). As a tool for evaluating temporal resolution ability in MS patients, these authors assess gap detection thresholds. For this purpose, participants had to detect silent intervals ranging from 2 to 20 ms embedded in 6-s segments of white noise. MS patients showed significantly higher thresholds for gap detection than healthy controls, indicating impaired auditory temporal resolution performance in MS patients.

Interval timing, including time estimation and duration discrimination, refers to the accurate timing of events. Accurate timing plays a crucial role for motor processes (16), speech (17), and learning (18) as well as working memory functioning (19). Hence, interval timing can be considered a basic component of cognitive functioning of all sorts [cf. (20)]. Given the important role of timing processes for cognitive functions shown to be impaired in MS patients, it is very surprising that no studies on interval timing in pediatric MS patients seem to exist. The aim of the present study, therefore, was to investigate, for the first time, performance on interval timing tasks in MS patients by comparing a group of pediatric MS patients with a group of healthy controls matched for age, sex, and psychometric intelligence.

The so-called distinct timing hypothesis [cf. (21, 22)] suggests two dissociable mechanisms for the timing of extremely brief durations in the subsecond range and longer durations, respectively. More precisely, interval durations less than approximately 300–500 ms can be perceived directly due to sensory-automatic temporal processing, whereas the duration of longer intervals needs to be reconstructed by higher mental processes [cf. (22)]. To tap into performance differences between MS patients and healthy controls in both the sensory-automatic as well as the cognitive processes involved in interval timing, two auditory duration-discrimination tasks with base durations of 100 and 1,000 ms, respectively, were applied in the present study. Furthermore, in order to control for more general, non-temporal, MS-related deficits in sensory transmission of acoustic stimuli [e.g., (23)], we also employed a frequency-discrimination task in addition to the two timing tasks.

METHODS

Participants

Twenty-three pediatric MS patients (19 females) participated in the present study. Their age ranged from 12 to 18 years ($M \pm SD$: 15.6 $\pm$ 1.9 years). Mean age at disease onset was 14.3 ($\pm$ 1.8) years, and the mean number of relapses was 2.61 ($\pm$ 1.03). Scores on the Expanded Disability Status Scale (EDSS; (24)) ranged from 0 to 6.5 with a mean score of 1.65 ($\pm$ 1.70), and their mean IQ was 97.43 ($\pm$ 9.37) according to Cattell's Culture Fair Test 20-R (CFT 20-R). Diagnoses were based on the recently revised McDonald

criteria (25). Twenty pediatric MS patients were treated with Interferon, two with Glatirameracetat, and one was therapy-naive. No patient received steroid treatment. Furthermore, no patient had clinical disease activity at the time of testing, and the attending doctors judged the clinical status of all participating patients as stable.

Previous research reveals that interval timing improves with increasing age of children and adolescents (26, 27) and that males might have lower discrimination thresholds than females (28). Furthermore, psychometric intelligence is positively related to performance on interval timing tasks (29) and has a differential effect on cognitive impairments due to MS (30). Therefore, the 23 MS patients were compared to 23 participants, out of a pool of 63 (neurologically and psychologically) healthy adolescents, matched for age, sex, and intelligence by means of a nearest-neighbor matching algorithm (31). The algorithm determined 19 female and four male healthy controls with a mean age of 16.4 ($\pm$ 2.2) years and a mean IQ of 99.4 ($\pm$ 10.7). They did not differ significantly from MS patients in age, $t(43.007) = 1.373$, $p = 0.177$, $d = 0.405$, and intelligence test scores, $t(43.210) = 0.658$, $p = 0.514$, $d = 0.194$.

All MS patients and healthy controls reported normal hearing and normal or corrected-to-normal vision. All participants and the parents of participants younger than 18 years were informed about the study protocol and signed informed consent prior to the study. The study was approved by the local ethics committee of the University of Witten/Herdecke (No. 173/2016).

Assessment of Depression

With the German Depression Inventory for Children and Adolescents [DIKJ; (32)], the severity of major depression symptoms was measured. For each of the 29 items, children chose the most applicable statement out of three alternatives. Stiensmeier-Pelster et al. (32) report high reliability coefficients ranging between Cronbach's $\alpha = 0.87$ and 0.92.

Assessment of Fatigue

With the 21 items of the German Modified Fatigue Impact Scale [MFIS; (33)], MS patients and healthy controls self-reported the severity with which fatigue affected physical, cognitive, and psychosocial aspects of their lives. According to Fisk et al. (33), the internal consistency is $\alpha = 0.81$. One MS patient did not respond to one and another patient did not respond to two MFIS items. Their sum scores were estimated on the basis of the other 20 or 19 items, respectively.

Expanded Disability Status Scale (EDSS)

The severity of MS-related disability in patients at the time of data collection was assessed by means of the EDSS (24). Scores could range from 0 to 10.

Experimental Tasks

The three experimental tasks employed in the present study have previously been validated for investigating interval timing and frequency discrimination in children and adolescents (26, 27). A Lenovo notebook (L540) was used with a 15" monitor as well as an external audio interface (Steinberg, UR22 MKII) and headphones (Sennheisser HDA300). Stimuli were presented by E-prime 2.0 experimental software and responses were given on a Cedrus® keyboard (RB-840).

Interval Timing in the Subsecond Range

Stimuli were white noise bursts presented at an intensity of 68 dB. The task consisted of 64 trials. Each trial consisted of a constant 100-ms standard interval and a variable comparison interval presented with an interstimulus interval (ISI) of 900 ms. The order of standard and comparison intervals within a trial was balanced and randomized across trials. The participant's task was to decide whether the first or the second stimulus was of longer duration by pressing one of two designated keys. Visual feedback was given after the response on the monitor for 1,500 ms (a "+" after a correct and a "–" after an incorrect response). After an intertrial interval of 600 ms, the next trial started.

The 64 trials were assigned to two interleaved series. In one series, the comparison (with an initial duration of 65 ms) was shorter than the standard interval. In the other series, the comparison (with an initial duration of 135 ms) was longer than the standard interval. Using the adaptive weighted up–down method (34), the difference between the comparison and standard intervals decreased after a correct response (5 ms in the first six trials, 3 ms in the following trials) and increased after an incorrect response (15 ms in the first six trials, 9 ms in the following trials). With this step-size ratio of 1:3, the two series converged to the 25% difference threshold (series with comparison interval shorter than standard) and the 75% difference threshold (series with comparison interval longer than standard), which were estimated from the last 20 trials of each series. The difference limen [DL; (35)] was computed as individual performance score, which refers to half the difference of the 75% and 25% difference thresholds. With this measure, superior performance on duration discrimination is indicated by smaller DL values.

Interval Timing in the One-Second Range

Hardware and software as well as the number of trials and the experimental procedure were the same as in the duration discrimination task in the range of milliseconds. The only differences were that the standard interval had a duration of 1,000 ms and the initial comparison intervals of 500 and 1,500 ms in the two series for the estimation of the 25% and the 75% difference thresholds. Step-sizes of the change of the comparison interval were 25 ms after a correct (100 ms in the first six trials) and 75 ms after an incorrect response (300 ms in the first six trials). Again, the DL was computed as individual performance score.

Frequency Discrimination

The experimental procedure was the same as for the duration-discrimination tasks with the following exceptions. All stimuli were sine wave tones of 500 ms duration and presented with an intensity of 68 db. Each trial consisted of a standard tone with a frequency of 440 Hz and a comparison tone with a variable frequency and initial values of 438 Hz in the series converging to the 25% difference threshold and 442 Hz in the series converging

to the 75% difference threshold. The step sizes were 0.3 Hz (0.5 Hz in the first six trials) after a correct response and 0.9 Hz (1.5 Hz in the first six trials) after an incorrect response. The ISI was 500 ms. The DL was computed as individual performance score.

Assessment of Intelligence

The CFT 20-R (36), composed of three subtests (series, classifications, matrices) with 27 items, respectively, and one subtest (topologies) with 20 items, was administered individually and lasted about 1 h. The reliability of the CFT 20-R is high with $r_{tt} = 0.96$. Originally, the CFT was developed to assess fluid intelligence as an abstract reasoning ability independent from crystallized intelligence, which refers to language- and knowledge-related abilities. Thus, rather specific language deficits in MS patients do not (or only marginally) bias the assessment of intelligence by means of the CFT. The high correlation between CFT scores and general intelligence underlines its adequacy to measure an individual's overall cognitive functioning (37). The version CFT 20-R (36) is validated for adults and children and comprises fine-grained age-stratified IQ norms for children older than 6 years, adolescents, and adults. As a dependent variable, correct responses across all subtests were added to raw scores and transformed to age-stratified IQ equivalents.

Time Course of the Study

The session started with verbal and written information about the study and signing informed consent by the participants and/or their parents followed by the administration of DIKJ and MFIS. The experimental part of the study started with two tasks, which lasted about 25 min and are reported in detail by Kapanci et al. (38). After a break of 15 min, the three discrimination tasks were presented in counterbalanced order. Each task lasted about 10 min. After another short break, participants completed the CFT 20-R. The total session lasted about 120 min.

RESULTS

An initial outlier detection revealed that discrimination thresholds in the interval timing task in the second range of one female MS patient and one female healthy control were more than three standard deviations above the mean of the respective group. These two participants were excluded from further analyses. Descriptive data as well as appropriate *t*-tests for age, IQ, depression, and fatigue are provided in **Table 1** for the remaining 22 MS patients and 22 healthy controls of the final sample. MS patients and healthy controls did not differ significantly in age and IQ. Furthermore, no significant differences were obtained regarding symptoms of depression and fatigue.

The main outcome variables of the present study were DL values in the two interval timing tasks (with stimuli in the subsecond and in the second range) and in the frequency discrimination task. Differences in discrimination performance, as indicated by DL values, between pediatric MS patients and healthy controls were investigated by means of three *t*-tests. In order to avoid alpha inflation, alpha was Bonferroni adjusted to $\alpha = 0.017$. Descriptive statistics, results of *t*-tests, and effect sizes (Cohen's *d*) are reported in **Table 1**. As can be seen from **Figure 1**, MS patients differed significantly from healthy controls in their performance on neither the frequency discrimination task (mean difference in DL = 0.4 Hz; 95% [−1.1, 1.9 Hz]) nor the interval timing task with stimulus durations in the one-second range (mean difference in DL = 26.9 ms; 95% CI [−14.2, 67.9 ms]). For interval timing in the subsecond range, however, mean DL was significantly larger in pediatric MS patients than in healthy controls. The mean difference in DL was 6.3 ms with the 95% confidence interval not including zero [1.7, 10.9 ms]. This result indicated worse performance in pediatric MS patients compared to healthy controls as they needed larger differences between two durations in the subsecond range to correctly identify the longer one.

It should be noted that the same pattern of results was obtained when only the data of the 18 female participants in each group were analyzed. Furthermore, neither in MS patients nor in healthy controls was age significantly correlated with performance on the interval timing or the frequency discrimination tasks. Given that the two groups did not differ in age, a systematic influence of age on the above reported results is unlikely.

DISCUSSION

The aim of the present study was to investigate possible impairments of interval timing in pediatric MS patients using two auditory duration discrimination tasks that focused on interval timing in the subsecond and one-second ranges, respectively. Compared to healthy controls, MS patients showed impaired interval timing in the subsecond range but no significant differences in the one-second range. These differences in the subsecond range are unlikely to be based on general deficits of auditory information processing as the auditory demands regarding the duration discrimination task in the one-second range were virtually identical. Moreover, there were no differences in the frequency discrimination thresholds between MS patients and healthy controls. Due to the matching procedure, differences in age, sex, and psychometric intelligence can also be excluded to explain MS patients' impaired interval timing in the subsecond range.

Our findings expand previous results on impaired perception of simultaneity and successiveness in adult MS patients in two ways. First, timing deficits do not only occur in adult but also in pediatric MS patients. Second, in addition to judgments of simultaneity and successiveness as previously reported (13–15) MS also affects interval timing in the subsecond range—at least in pediatric patients.

Our findings support the distinct timing hypothesis (21, 22), which suggests two dissociable mechanisms underlying the timing of extremely brief durations in the subsecond range and longer durations in the second range. It appears that the sensory-automatic temporal processing of extremely brief durations below 300–500 ms is substantially impaired in pediatric MS patients, whereas cognitively mediated temporal processing of longer durations is less affected.

TABLE 1 | Mean (M) and standard deviation (SD) of age, normed CFT 20-R IQ scores, fatigue (MFIS), and depression scores (DIKJ) as well as difference limen (DL) in the two interval timing tasks and the frequency discrimination task for 22 pediatric MS patients and 22 healthy controls.

	Pediatric MS		Healthy controls		*t*	*df*	*P*	*d*
	M	*SD*	*M*	*SD*				
Age [years]	15.5	1.9	16.5	2.1	−1.644	41.635	0.108	−0.496
IQ	97.1	9.4	99.5	11.0	−0.781	41.083	0.440	−0.235
MFIS	30.7	18.1	23.5	10.5	1.632	33.623	0.112	0.492
DIKJ	13.2	6.0	12.8	6.3	0.246	41.875	0.807	0.074
DL of interval timing in the millisecond range [ms]	19.8	8.3	13.6	6.8	2.753	40.308	0.009	0.830
DL of interval timing in the second range [ms]	166.6	67.4	139.7	67.4	1.322	42.000	0.193	0.399
DL of frequency discrimination [Hz]	6.4	2.0	6.0	2.9	0.525	37.756	0.603	0.158

Also reported are t-tests and corresponding effect sizes (Cohen's d).

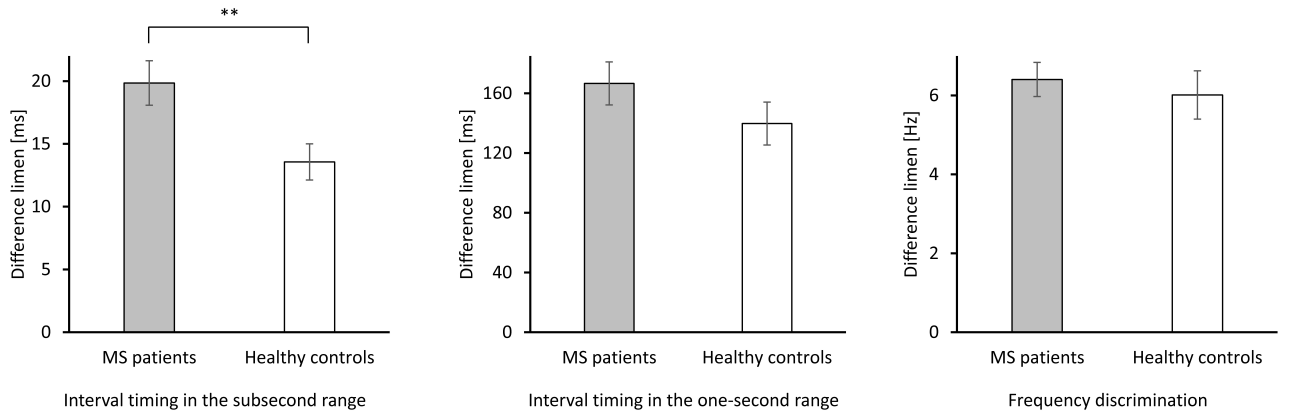

FIGURE 1 | Performance as indicated by DL of 22 pediatric MS patients and 22 healthy controls in the two duration discrimination tasks (left panel: interval timing in the subsecond range; intermediate panel: interval timing in the one-second range) and in the frequency discrimination task (right panel). Smaller DL indicate better performance on the respective task. $^{**}p < 0.01$ (two-tailed).

As depicted in **Figure 1**, MS patients show a performance decrement in both duration discrimination tasks compared to healthy controls. We cannot rule out that, in a larger sample, the difference in interval timing in the one-second range between MS patients and healthy controls might also have become statistically significant. The effect size, however, was more than twice as large for the interval timing in the subsecond compared to the one-second range. Thus, the sensory-automatic processes underlying the timing of intervals in the subsecond range seem to be particularly vulnerable to degenerative changes in the brain associated with MS.

There is good empirical evidence for the notion that distinct but partly overlapping neural networks underlie interval timing in the sub- and suprasecond range. In the meta-analysis by Wiener et al. (39), activation in the inferior frontal cortex, supplementary motor areas, precental gyrus, parietal lobe, insular cortex, claustrum, and putamen was related to both sub- and suprasecond timing. Particularly pronounced activation during temporal processing in the suprasecond range was found for the (right) prefrontal brain areas [see also (40)]. For timing in the subsecond range, specific activation was primarily identified in subcortical areas, such as the cerebellum (39, 41), thalamus, and striatal parts of the basal ganglia (39, 42) as well as some neocortical areas (e.g., the right inferior parietal lobe). Most interestingly, MS-related deficits in subcortical areas have been reported even at an early stage of the disease (43) and more frequently in pediatric than adult patients (44). Hence, a tentative explanation of the present findings might be that pediatric MS patients' impaired timing performance in the subsecond range is indicative of deficits in subcortical brain areas.

Previous research shows that accurate timing in the subsecond range plays an important role for motor coordination and visuomotor integration (16) and for speech perception and production (17) as well as speed of information processing (29). Against this background, it is particularly interesting that pediatric MS patients process information more slowly than healthy controls (5), have more problems integrating visuomotor information (1, 3, 45–47), and have deficits in fine motor coordination (46). Moreover, pediatric MS patients more frequently show receptive and expressive language deficits (1, 45, 47, 48). Thus, it would be promising for future research to investigate to what degree pediatric MS patients' timing deficits—as observed in the present study—is functionally related to their commonly observed deficits in motor coordination,

processing speed, and speech. Such results would contribute to a better understanding of the neurocognitive mechanisms underlying MS patients' health-related restrictions observed in everyday life.

In sum, pediatric MS patients in the present study show impaired performance on interval timing in the subsecond range compared to healthy controls. This impairment is unlikely to be explained by auditory deficits because no performance differences between the two groups could be established for interval timing in the one-second range and frequency discrimination. As most brain areas specifically affecting interval timing in the subsecond range are subcortical, a tentative, but plausible explanation might point to subcortical alterations in the present sample of pediatric MS patients. Timing in the subsecond range is important for many daily life activities, such as visuomotor coordination or speech commonly impaired in pediatric MS patients. If future studies establish functional relationships between MS-related deficits in interval timing in the subsecond range and these daily life activities, the investigation of interval timing in the subsecond range might be a promising approach to better understand the underlying causes of these deficits.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by local ethic committee of the University of Witten/Herdecke (No. 173/2016). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

ST, TK, and KR conceptualized and planned the experiments. ST, TK, CK, MH, TG, MS, CE, JK, CT, and KR carried out the experiments. ST, TK, and CK contributed to sample preparation. ST, TK, CK, and TR contributed to the interpretation of the results. ST took the lead in writing the manuscript. All authors were involved in recruiting participants, provided critical feedback, and revised the manuscript.

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Impact of Cognitive Reserve and Structural Connectivity on Cognitive Performance in Multiple Sclerosis

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Background: Cognitive reserve (CR) could attenuate the impact of the brain burden on the cognition in people with multiple sclerosis (PwMS).

Objective: To explore the relationship between CR and structural brain connectivity and investigate their role on cognition in PwMS cognitively impaired (PwMS-CI) and cognitively preserved (PwMS-CP).

Methods: In this study, 181 PwMS (71% female; 42.9 ± 10.0 years) were evaluated using the Cognitive Reserve Questionnaire (CRQ), Brief Repeatable Battery of Neuropsychological tests, and MRI. Brain lesion and gray matter volumes were quantified, as was the structural network connectivity. Patients were classified as PwMS-CI (z scores = −1.5 SD in at least two tests) or PwMS-CP. Linear and multiple regression analyses were run to evaluate the association of CRQ and structural connectivity with cognition in each group. Hedges's effect size was used to compute the strength of associations.

Results: We found a very low association between CRQ scores and connectivity metrics in PwMS-CP, while in PwMS-CI, this relation was low to moderate. The multiple regression model, adjusted for age, gender, mood, lesion volume, and graph metrics (local and global efficiency, and transitivity), indicated that the CRQ ($\beta = 0.26$, 95% CI: 0.17–0.35) was associated with cognition (adj $R^2 = 0.34$) in PwMS-CP (55%). In PwMS-CI, CRQ ($\beta = 0.18$, 95% CI: 0.07–0.29), age, and network global efficiency were independently associated with cognition (adj $R^2 = 0.55$). The age- and gender-adjusted association between CRQ score and global efficiency on having an impaired cognitive status was −0.338 (OR: 0.71, $p = 0.036$) and −0.531 (OR: 0.59, $p = 0.002$), respectively.

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Conclusions: CR seems to have a marginally significant effect on brain structural connectivity, observed in patients with more severe clinical impairment. It protects PwMS from cognitive decline regardless of their cognitive status, yet once cognitive impairment has set in, brain damage and aging are also influencing cognitive performance.

Keywords: cognitive reserve, structural connectivity, graph theory, cognition, multiple sclerosis

INTRODUCTION

Cognitive impairment (CI) has been reported in 40–70% of people with multiple sclerosis (PwMS) (1) and it has a negative impact on their quality of life (2). It is associated with the combined effect of both white matter (WM) and gray matter (GM) damage (3). However, magnetic resonance imaging (MRI) metrics like lesion volume (Lv) or GM volume (GMv) only partially explain the cognitive changes of PwMS. Non-conventional MRI techniques, such as diffusion-weighted imaging (DWI) or functional MRI (fMRI), can be used to further explore structural and functional brain connectivity and its associations with CI (4, 5). Moreover, through theoretical graph analysis, it has been suggested that disrupting the optimal balance between local integration and global segregation of network components might hamper information flow, exerting a negative impact on cognition (5, 6).

Some individuals better maintain their cognitive performance despite the presence of substantial brain damage. This clinico-pathological dissociation (7) indicates that certain factors protect against cognitive decline, such as the cognitive reserve (CR), understood to be lifelong intellectual enrichment that attenuates the negative effect of MS disease burden on neuropsychological activity (8, 9). Previous studies in MS and other neurodegenerative diseases, such as Alzheimer's disease, suggested that patients with higher CR displayed better cognitive function regardless of having similar brain damage (7, 10, 11). Indeed, CR seems to preserve brain network functional connectivity counterbalancing the impact of the disruption of WM tracts due to lesions in MS on cognition (12). However, this protective role of the CR diminishes over the MS disease course as the brain burden becomes stronger (13, 14). Before the appearance of CI, the brain probably employs adaptive and compensatory mechanisms, undergoing structural and functional reorganization in response to the pathological changes caused by MS (5, 15). However, the accumulation of brain damage can lead to network dysfunction that may contribute significantly to the development of CI in PwMS (16). As far as we know, the relationship between CR and structural brain connectivity remains unexplored.

We hypothesized that individuals with higher CR would exhibit higher structural connectivity and, consequently, better cognitive performance. Also, the influence of CR on cognitive performance in PwMS may be distinct before and after the emergence of CI. Therefore, we aimed to understand the association between CR and structural connectivity integrity and their impact on cognition in PwMS. For this, we analyzed their role in patients with different cognitive status, thus in PwMS cognitively impaired (PwMS-CI) and in those who remained cognitively preserved (PwMS-CP).

MATERIALS AND METHODS

Participants

A cohort of 181 PwMS (aged 18–65 years) who fulfilled the 2010 McDonald criteria (17) was consecutively selected at the MS Unit of the Hospital Clinic of Barcelona. To be included, patients had to be free from relapses in the last 30 days and have no significant neurological or psychiatric condition that could interfere with cognitive functioning. In this cross-sectional study, patients were evaluated using clinical and cognitive scales, and they underwent an MRI scan. We collected data regarding MS type, disease duration, current treatment, and global disability, the latter measured using the Expanded Disability Status Scale (EDSS) (18). In addition, a global score of depression and anxiety symptoms was obtained for the patients using the Hospital Anxiety and Depression Scale (HADS) (19). The Ethics Committee at the Hospital Clinic of Barcelona approved the study and all the participants signed an informed consent form prior to their enrollment on the study.

Neuropsychological Assessment

Cognition was assessed using the Brief Repeatable Battery of Neuropsychological tests (BRB-N) (20). This battery includes different tests assessing cognitive domains as follows: (1) verbal learning and memory: Selective Reminding Test (SRT, with two subtests: consistent long-term retrieval as an indicator of consolidation, and delayed retrieval); (2) visuospatial learning and memory: 10/36 Spatial Recall Test (SPART, with two subtests: immediate retrieval and for delayed retrieval); (3) attention, working memory, and information processing speed: Symbol Digit Modalities Test (SDMT) and Paced Auditory Serial Addition Test (PASAT) 3 s per digit version; and (4) verbal fluency and cognitive flexibility: Word List Generation (WLG).

We first calculated z scores for all BRB-N tests, using demographically adjusted (age and education) regression models according to the normative data published in the Spanish population (21), classifying patients as PwMS-CI or PwMS-CP. Patients were classified as PwMS-CI if performance was below $z = -1.5$ standard deviations (SD) of the norm in at least two cognitive tests of the same or different cognitive domain. In addition, raw values were transformed into z scores (zBRB) by subtracting the mean and dividing by the SD of the whole sample in order to obtain a mean score of cognitive performance, avoiding the educational effect related to CR and the aging effect on cognition.

Assessment of CR

CR was assessed using the Cognitive Reserve Questionnaire (CRQ) (22), a standardized scale in which higher scores represent higher levels of CR (maximum 25 points). This test is composed of eight items that measure different intellectual enrichment factors, including the individual's education, their parent's education, training courses, occupation, musical training, language studies, reading activity, and intellectual games in which they have participated during their adult lifetime. Items do not contemplate a specific period, thus addressing experiences throughout life (23). This questionnaire was administered by an experienced neuropsychologist before the cognitive assessment. The CRQ has been previously applied to both healthy elderly and diseased populations (24, 25).

Magnetic Resonance Images

MRI Acquisition

MR images were acquired on a 3-Tesla Magnetom Trio (SIEMENS, Erlanger, Germany) scanner using a 32-channel phased-array head coil. The protocol applied involved a 3D-Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE), 3D-T2 fluid-attenuated inversion recovery (FLAIR), and DWI sequences (see **Supplementary Material** for a detailed description of the sequences).

Structural MRI Processing for Volumetric Analysis

WM lesions were defined semi-automatically on the 3D-MPRAGE sequence using the Jim7 software (http://www.xinapse.com/j-im-7-software/). To improve MS lesion identification, the co-registered 3D-FLAIR image was used as a reference. Thereafter, lesion in-painting was applied to the 3D-MPRAGE image to enhance segmentation and registration in PwMS (26). The FSL and SIENAX tools (27) were used to obtain the normalized Lv and GMv (nLv and nGMv).

Whole Brain Structural Connectivity Reconstruction

Cortical parcellation was performed with the Mindboggle software (28) using a cortical labeling parcellation scheme from FreeSurfer (https://surfer.nmr.mgh.harvard.edu/) that is based on the Desikan-Killiany atlas (29). Subcortical GM structures were segmented by applying the FIRST tool (fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST). Thirty-one cortical regions and seven subcortical GM structures per hemisphere were used as nodes of the network.

DWI processing was performed as described previously (5, 30). High Angular Resolution Diffusion Imaging (HARDI) images were denoised and corrected for geometric distortions and head motion (31). The structural connectome was obtained using multi-tissue constrained spherical deconvolution-based tractography, applying the second-order integration over fiber orientation distributions and an anatomically constrained tractography framework (32), available in the MRtrix3 software package (http://www.mrtrix.org/). WM and lesion masks were registered to the undistorted HARDI images by applying boundary-based registration (33). Fiber tracking required a seeding mask that corresponded to the normal-appearing WM and MS lesions, thereby avoiding premature cessation of the reconstruction in areas with a more complex structural

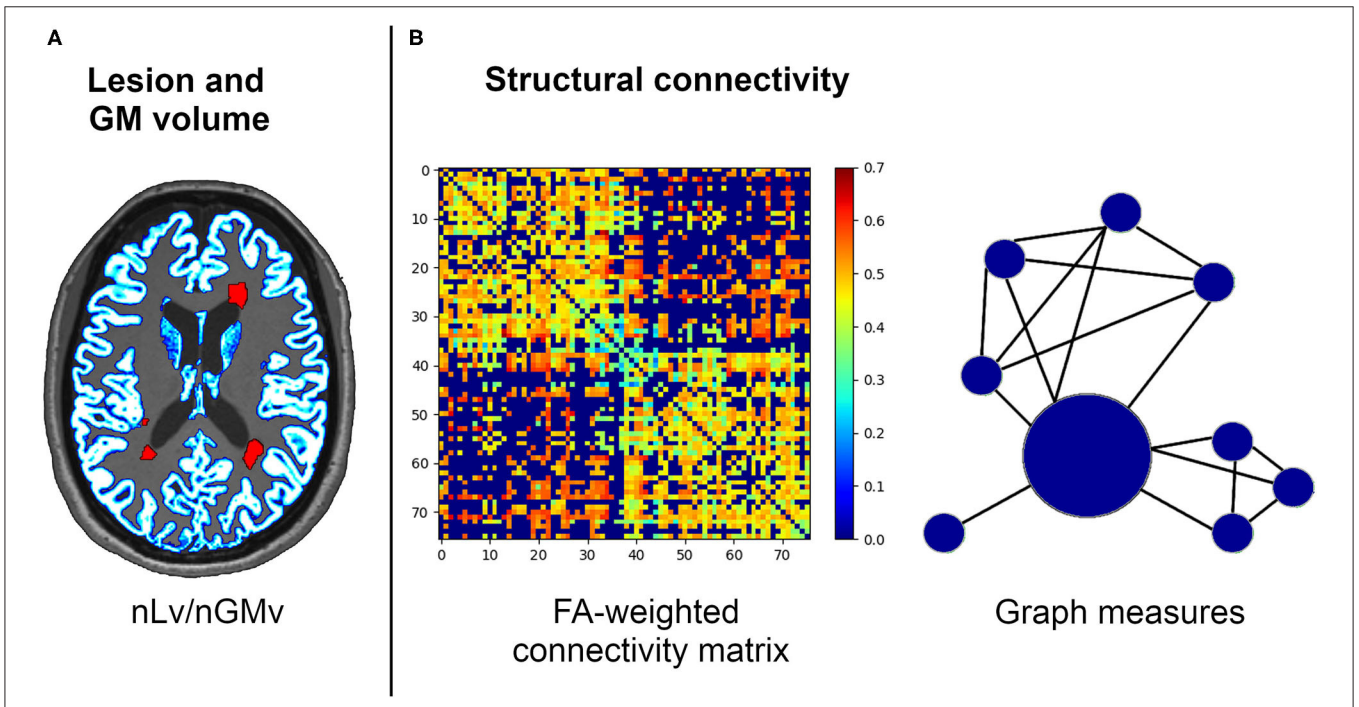

FIGURE 1 | Structural connectivity framework for the neuroimaging processing of the volumetric **(A)** and network **(B)** analysis. FA, fractional anisotropy; nGMv, normalized gray matter volume; nLv, normalized lesion volume.

TABLE 1 | Demographic, clinical, and MRI data of the study population.

	Entire cohort (n = 181)	Cognitive status groups		
		PwMS-CP (n = 100)	PwMS-CI (n = 81)	p-value
Demographic data				
Female, *n* (%)	128 (71)	76 (76)	52 (64)	0.116[a]
Age (years)	42.9 (10.1)	41.4 (9.0)	44.7 (11.0)	0.027[c]
CRQ score median [IQR]	16 (12–19)	17 (11–18)	15 (11–18)	0.014[b]
Education level				
Basic (0–8 years)	11 (6)	6 (6)	5 (6)	0.036[a]
Primary (9–12 years)	76 (42)	40 (40)	36 (45)	
Secondary (13–16 years)	58 (32)	40 (40)	18 (22)	
Higher (>17 years)	36 (20)	14 (14)	22 (27)	
Right handed	158 (87)	88(88)	70 (86)	0.523[a]
Clinical data				
Type of MS, *n* (%)				
RRMS	166 (92)	96 (96)	70 (86)	0.040[b]
SPMS	15 (8)	4 (4)	11 (14)	
Disease duration (years)	10.3 (9.2)	9.3 (9.0)	11.6 (9.3)	0.132[b]
EDSS score, median (range)	2.0 (0–6.5)	2.0 (0–6.5)	2.0 (0–6.5)	0.014[b]
Current use of DMT, *n* (%)	147 (81.2)	84 (84)	63 (77.8)	0.382[b]
zBRB	0.01 (0.71)	0.42 (0.48)	−0.51 (0.61)	<0.001[b]
HADS score, median [IQR]	9 (5–15)	8 (4–14)	10 (6–16)	0.125[b]
Neuroimaging data				
nLv (cm^3)	9.43 (12.82)	6.32 (7.22)	13.27 (16.69)	<0.001[b]
nGMv (cm^3)	782.35 (61.14)	794.43 (52.29)	767.44 (67.96)	0.003[c]
Nodal strength	11.68 (1.69)	12.140 (1.37)	11.110 (1.88)	<0.001[b]
Local efficiency	0.363 (0.02)	0.367 (0.02)	0.359 (0.03)	0.033[b]
Cluster coefficient	0.261 (0.02)	0.264 (0.01)	0.257 (0.02)	0.027[b]
Transitivity	0.245 (0.02)	0.249 (0.02)	0.241 (0.02)	0.007[b]
Global efficiency	0.289 (0.02)	0.295 (0.02)	0.282 (0.03)	0.003[b]
Assortativity	0.012 (0.03)	0.008 (0.03)	0.016 (0.03)	0.053[b]

The data represent the absolute numbers and proportions of the qualitative data, and the mean and SD for the quantitative data, unless otherwise specified. IQR, interquartile range; PwMS-CP/CI, patients with multiple sclerosis cognitively preserved/cognitively impaired; CRQ, Cognitive Reserve Questionnaires; RRMS, relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; EDSS, Expanded Disability Status Scale; DMT, disease-modifying treatment; zBRB, global cognitive performance score; HADS, Hospital Anxiety and Depression Scale; nLv, normalized lesion volume; nGMv, normalized gray matter volume.

[a]*Chi-squared test;* [b]*Wilcoxon–Mann–Whitney test;* [c]*Student's t-test.*

architecture and with low fractional anisotropy (FA) (30). Anatomical exclusion criteria were applied to minimize the number of anatomically aberrant connections originated from the tractography procedure (30). Finally, the total 76 segmented cortical and subcortical regions were used to define the nodes of the network, and matrices were generated to represent the mean FA values of the connections.

Network Analysis

Graph theory metrics were computed using the Brain Connectivity Toolbox (https://sites.google.com/site/bctnet). Graph metrics were analyzed to express the global connectivity properties of the network, including the nodal strength (the sum of weights connected to the node); measures of segregation, such as the local efficiency (the average of the inverse of the shortest path length in the network computed on node neighborhoods), the clustering coefficient (the fraction of a node's neighbors that are neighbors of each other), and transitivity (the ratio of triangles to triplets in the network); integration, as measured through the global efficiency (the average inverse shortest path length in the whole network); and brain resilience, reflected by assortativity (a correlation coefficient of the degrees of separation of all the nodes at two opposite ends of a link) (34). A representative image of the MRI metrics used in this study is presented in **Figure 1**.

Statistical Analysis

All demographic, clinical, neuropsychological, MRI markers of brain burden, and connectivity values were described through the mean and SD, and by the absolute numbers and the proportions for quantitative and qualitative data, respectively. The normality of continuous data was checked using histograms and appropriate statistical methods as the Shapiro–Wilks test. We compared the aforementioned characteristics between PwMS-CI and PwMS-CP patients using a Chi-squared test, a Wilcoxon–Mann–Whitney U-test or a Student's t-test, depending on the data distribution.

Age- and gender-adjusted linear regressions were done to analyze the associations between CRQ and cognition, between structural connectivity and cognition, and between CRQ and structural connectivity on the entire cohort and in each group of PwMS separately. To understand the role of CR and structural connectivity on cognitive performance in the context of other demographic and MS-related factors, we fitted a multiple regression model that included relevant demographic and clinical variables. Variables were standardized using the mean and SD: CRQ score, age, gender, EDSS, HADS score, nLv, nGMv, and graph measures of segregation, integration, and brain resilience (nodal strength, global and local efficiency, clustering coefficient, transitivity, and assortativity). The Akaike Information Criterion (AIC) was then used to select the variables that best fit a model based on the whole cohort. As the main objective of the study was to determine the influence of CR and structural connectivity on cognitive performance in patients with different cognitive status, we applied the same multiple regression model separately in PwMS-CI and PwMS-CP, with the variables selected from the AIC. We computed the strength of the associations using Hedges' g effect size.

Furthermore, an age- and gender-adjusted logistic regression analysis was used to estimate the odds ratio (OR) of having an impaired cognitive performance associated with the increase per unit of the CR and MRI connectivity metrics associated to cognition in the multiple regression model.

Statistical analyses were performed with R statistical software (version 3.6.0, www.R-project.org), setting the level of

significance at $p < 0.05$ and correcting multiple comparisons for the false discovery rate (FDR).

RESULTS

This study was carried out on a population of 181 PwMS who were mostly female (71%), middle-aged adults (42.9 ± 10.0 years), and who had a median CRQ score of 16 (interquartile range, IQR: 12–24). In the cohort, 81 patients (45%) were classified as PwMS-CI, and the remaining 100 patients (55%) were considered PwMS-CP. The group of PwMS-CI more frequently presented with a secondary progressive phenotype of the disease, with lower CRQ scores and with higher EDSS scores. They also presented worse volumetric and connectivity measures in images than PwMS-CP, although assortativity was no different (see **Table 1** for further details).

Relationship Between CR, Structural Connectivity, and Cognition

We found significant associations between CRQ and zBRB scores in the entire cohort (β = 0.324, 95% confidence interval, CI: 0.24–0.41, $p < 0.001$), in PwMS-CP (β = 0.253, 95% CI: 0.17–0.34, $p < 0.001$; Hedges' *g*: 0.521, 95% CI: 0.22–0.82), and also in PwMS-CI (β = 0.300, 95% CI: 0.19–0.41, $p < 0.001$; Hedges' *g*: 0.626, 95% CI: 0.33–0.93). In parallel, significant associations were also found between graph structural connectivity properties and cognitive scores in the entire cohort and in the PwMS-CI group in all studied graph measures except for assortativity

TABLE 2 | Associations between graph structural connectivity properties and cognition in both PwMS groups.

	PwMS-CP (*n* = 100)			PwMS-CI (*n* = 81)		
	β (95% CI)	**Hedges' *g* (95% CI)**	***p*-value**	**β (95% CI)**	**Hedges' *g* (95% CI)**	***p*-value**
Nodal strength	0.023 (−0.10 to 0.15)	0.046 (−0.25 to 0.34)	0.710	0.282 (0.18 to 0.38)	0.585 (0.29 to 0.89)	<0.001
Local efficiency	0.029 (−0.09 to 0.14)	0.058 (−0.24 to 0.35)	0.710	0.230 (0.13 to 0.33)	0.471 (0.17 to 0.77)	<0.001
Cluster coefficient	0.024 (−0.09 to 0.14)	0.048 (−0.25 to 0.34)	0.710	0.218 (0.11 to 0.32)	0.445 (0.15 to 0.74)	<0.001
Transitivity	0.030 (−0.08 to 0.15)	0.06 (−0.23 to 0.35)	0.710	0.225 (0.12 to 0.33)	0.460 (0.16 to 0.76)	<0.001
Global efficiency	0.032 (−0.09 to 0.15)	0.064 (−0.23 to 0.36)	0.710	0.268 (0.17 to 0.37)	0.554 (0.26 to 0.85)	<0.001
Assortativity	0.059 (−0.04 to 0.16)	0.118 (−0.18 to 0.41)	0.710	−0.101 (−0.23 to 0.03)	−0.202 (−0.5 to 0.09)	0.132

Beta coefficients and 95% confidence intervals (CI) from age- and gender-adjusted linear regression models. PwMS-CP/CI, patients with multiple sclerosis cognitively preserved/cognitively impaired. P-values were adjusted by FDR.

TABLE 3 | Associations between CRQ and graph structural connectivity properties in both PwMS groups.

	PwMS-CP (*n* = 100)			PwMS-CI (*n* = 81)		
	β (95% CI)	**Hedges' *g* (95% CI)**	***p*-value**	**β (95% CI)**	**Hedges' *g* (95% CI)**	***p*-value**
Nodal strength	−0.072 (−0.24 to 0.10)	−0.144 (−0.48 to 0.15)	0.490	0.267 (0.03 to 0.50)	0.552 (0.25 to 0.85)	0.068*
Local efficiency	−0.114 (−0.29 to 0.06)	−0.229 (−0.52 to 0.07)	0.412	0.200 (−0.05 to 0.45)	0.407 (0.11 to 0.70)	0.136
Cluster coefficient	−0.080 (−0.26 to 0.10)	−0.160 (−0.45 to 0.13)	0.490	0.187 (−0.06 to 0.43)	0.379 (0.08 to 0.67)	0.136
Transitivity	−0.056 (−0.24 to 0.13)	−0.112 (−0.41 to 0.18)	0.547	0.258 (0.02 to 0.50)	0.532 (0.23 to 0.83)	0.068*
Global efficiency	−0.113 (−0.28 to 0.06)	−0.226 (−0.52 to 0.07)	0.412	0.254 (0.01 to 0.50)	0.523 (0.23 to 0.82)	0.068*
Assortativity	0.172 (−0.03 to 0.37)	0.348 (0.05 to 0.64)	0.412	0.239 (0.02 to 0.46)	0.490 (0.19 to 0.79)	0.068*

Beta coefficients and 95% confidence intervals (CI) from age- and gender-adjusted linear regression models. PwMS-CP/CI, patients with multiple sclerosis cognitively preserved/cognitively impaired. P-values were adjusted by FDR.
**P < 0.05 before correcting for multiple comparisons by FDR.*

TABLE 4 | Associations between clinical and MRI variables and cognitive performance in both PwMS groups.

Parameters	PwMS-CP ($n = 100$)			PwMS-CI ($n = 81$)		
	β (95% CI)	Hedges' g (95% CI)	p-value	β (95% CI)	Hedges' g (95% CI)	p-value
CRQ score	0.259 (0.17 to 0.35)	0.534 (0.24 to 0.83)	<0.001	0.179 (0.07 to 0.29)	0.362 (0.07 to 0.66)	0.012
Age	−0.097 (−0.20 to 0.00)	−0.194 (−0.49 to 0.10)	0.165	−0.119 (−0.21 to −0.02)	−0.239 (−0.53 to 0.06)	0.042
Gender	0.198 (−0.01 to 0.41)	0.402 (0.106 to 0.70)	0.165	0.179 (−0.03 to 0.39)	0.362 (0.07 to 0.66)	0.121
HADS score	−0.083 (−0.18 to 0.01)	−0.166 (−0.46 to 0.13)	0.165	−0.079 (−0.18 to 0.02)	−0.158 (−0.45 to 0.14)	0.141
Local efficiency	0.087 (−0.19 to 0.37)	0.174 (−0.12 to 0.48)	0.585	−0.084 (−0.38 to 0.21)	−0.168 (−0.46 to 0.13)	0.567
Transitivity	−0.156 (−0.42 to 0.11)	−0.315 (−0.61 to −0.02)	0.404	−0.284 (−0.56 to 0.01)	−0.590 (−0.89 to −0.29)	0.087
Global efficiency	0.165 (−0.17 to 0.50)	0.333 (0.04 to 0.63)	0.440	0.504 (0.18 to 0.83)	1.162 (0.85 to 1.48)	0.012
nLv (cm^3)	0.047 (−0.12 to 0.22)	0.094 (−0.20 to 0.39)	0.585	−0.103 (−0.21 to 0.00)	−0.206 (−0.45 to 0.09)	0.094

Beta coefficients and 95% confidence intervals (CI) from a multiple linear regression model. PwMS-CP/CI, patients with multiple sclerosis cognitively preserved/cognitively impaired; CRQ, Cognitive Reserve Questionnaires; HADS, Hospital Anxiety and Depression Scale; nLv, normalized lesion volume. P-values were adjusted by FDR.

(entire PwMS cohort β between 0.215 and 0.285, 95% CI: 0.12–0.38, $p < 0.001$) (**Table 2**).

Regarding the relationship between the CRQ and structural connectivity, we found a very low effect size association in PwMS-CP and a low-to-moderate correlation in PwMS-CI. However, after multiple comparisons, those associations did not reach statistical significance ($p < 0.05$). Nodal strength, transitivity, and global efficiency were the metrics showing a moderate effect size in this group (**Table 3**).

Models to Explain Cognitive Performance

Based on the AIC, the final multiple linear regression model included CRQ score, age, gender, the HADS score, global and local efficiency, transitivity, and nLv as variables associated with cognitive performance. This model was applied to each group of patients separately. In PwMS-CP, 34% of the cognitive performance (mean zBRB score) was explained by the model (adj $R^2 = 0.34$, $p < 0.001$). In this group, a one-point increase in the CRQ score was associated with a 0.26-point increase in the zBRB (the only significant variable in the model) (β = 0.259, 95% CI: 0.17–0.35, $p < 0.001$). The CRQ showed a moderate association with cognitive performance (Hedges' $g = 0.534$, 95% CI: 0.24–0.83). In the PwMS-CI, 55% of the variability in the zBRB was explained by the model (adj $R^2 = 0.55$, $p < 0.001$). In these patients, a one-point increase in the CRQ score was associated with a 0.18-point increase in the zBRB (β = 0.179, 95% CI: 0.07–0.29, $p = 0.012$). Moreover, in this model age (β = −0.119, 95% CI: −0.21 to −0.02, $p = 0.041$) and global efficiency (β = 0.504, 95% CI: 0.18–0.83, $p = 0.012$) were significantly associated negatively and positively with the zBRB, respectively (**Table 4** and **Figure 2**). Transitivity and global efficiency were the variables showing a moderate and high association with cognition (transitivity Hedges' $g = -0.590$, 95% CI: −0.89 to −0.29 and global efficiency Hedges' $g = 1.162$, 95% CI: 0.85–1.48; **Table 4**).

We evaluated the predictive value of CRQ and global efficiency on cognitive status. The age- and gender-adjusted association between CRQ score and global efficiency on having an impaired cognitive status was −0.338 (OR: 0.71, 95% CI: 0.52–0.97, $p = 0.036$) for CRQ score and −0.531 (OR: 0.59, 95% CI: 0.41–0.82, $p = 0.002$) for global efficiency.

DISCUSSION

In this study, we set out to understand the protective effect of CR on structural network integrity and their impact on cognition in PwMS in relation to other important demographic and MS-related factors. As such, we explored the relationship between CR and structural brain connectivity and analyzed different determinants of neuropsychological performance, including clinical information and some metrics of brain burden, in the presence or absence of CI. Although we found a marginal association between CR and structural connectivity integrity, it is only after brain damage reaches a significant level and CI is present that we found a moderate association between these measures. While the CR is the only variable associated with cognition in patients with good cognitive performance, when CI flourishes, structural brain damage, and aging are also related to this parameter. Indeed, in PwMS-CI, the impact of network integrity dysfunction is stronger than the effect of lifelong intellectual enhancement. The observed benefit of CR on cognitive performance has practical implications, including the implementation of strategies for intellectual life enrichment in addition to conventional therapies to palliate the effect of brain damage.

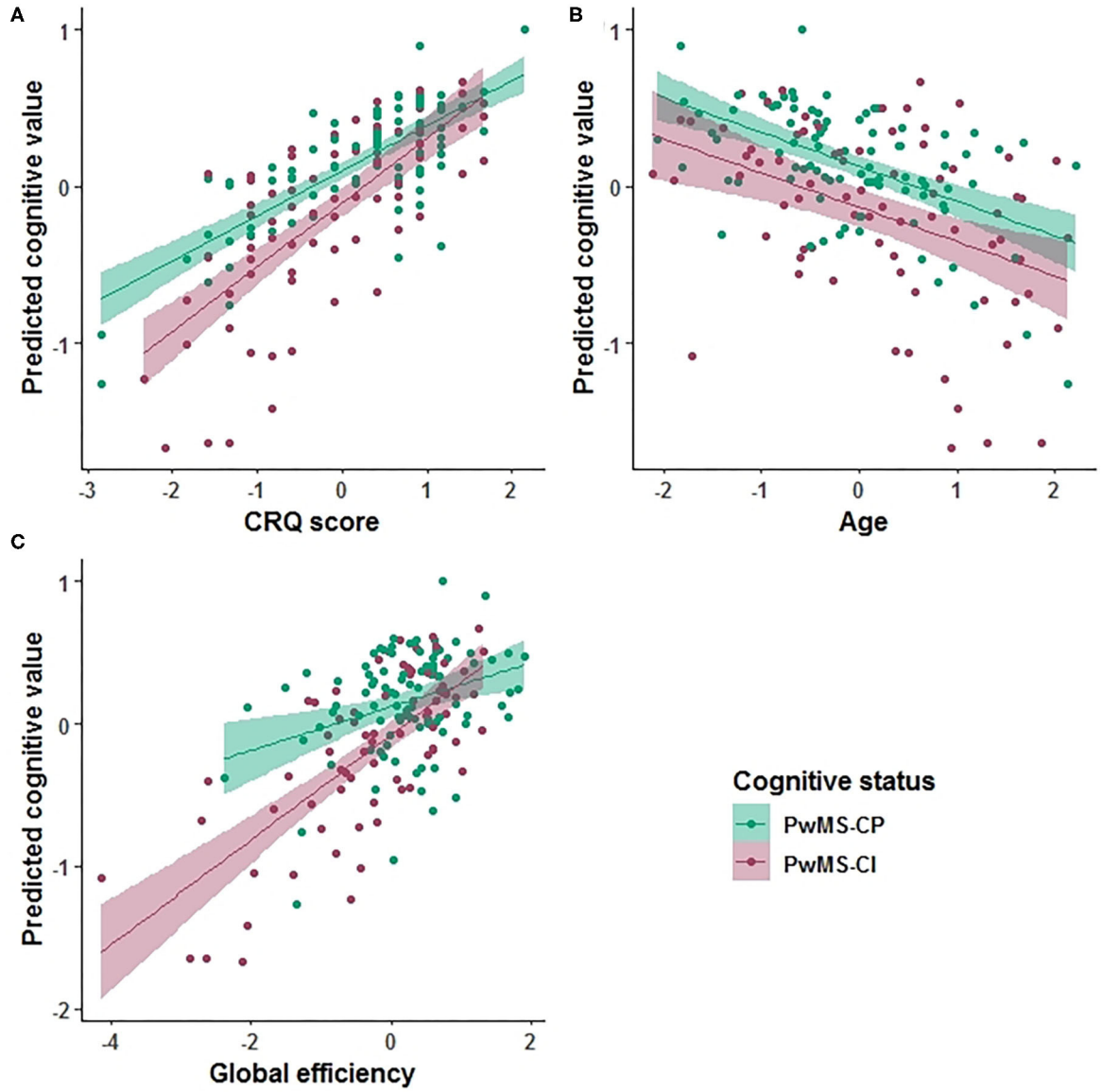

FIGURE 2 | Prediction value in the cognitive explanatory model. Marginal effects of the Cognitive Reserve Questionnaire (CRQ) score **(A)**, age **(B)**, and global efficiency **(C)** are shown. The PwMS-CP group is colored green and the PwMS-CI is represented in red.

The relationship between CR and cognition has been studied in several neurological diseases, including MS (7). It has been suggested that more intellectual enrichment potentially protects PwMS from cognitive decline (7, 14, 35). Indeed, we found that higher scores in the CRQ scale were associated with better cognitive performance, meaning that CR could help to preserve the cognitive function. However, to the best of our knowledge, the effect that CR may have on structural connectivity networks remains unknown. In this regard, we observed a relationship between structural brain connectivity dysfunction and CRQ scores with low-to-moderate effect sizes. Specifically, in the PwMS-CI group, lower scores of CRQ were associated with decreased nodal strength, transitivity, and global efficiency. Thus, CR might have a positive effect on the integration mechanisms that support long-range connections (36) and on network segregation, reflecting compensatory mechanisms against cerebral damage. Other studies focusing on functional networks found links between CR and network efficiency in healthy elderly individuals (15) and in PwMS (12), which makes the protective role of CR more plausible on functional than on structural connectivity. Considered together, a higher CR tends to ameliorate the negative impact of MS on brain connectivity and seems to protect against cognitive decline.

Investigating the interaction between CR and structural connectivity on cognitive performance, we demonstrate the protective effect of intellectual life enrichment assessed with the

CRQ on cognition in PwMS, with and without CI, irrespective of age, mood disorders, and brain burden. More specifically, we studied CR and structural connectivity integrity along with clinical and more conventional MRI parameters of brain damage in a model that explains cognitive performance in patients with different cognitive status. In the PwMS-CP group, CR explains the 34% of the variance in neuropsychological performance, whereas in the PwMS-CI group, CR together with age and global efficiency explain the 55% of the variance in cognitive performance. In this latter group, the association between CR and cognition was weaker than in the PwMS-CP cohort. The link between aging and structural brain connectivity with cognition in PwMS-CI was expected, since, as patients get older and brain damage due to pathological events accumulates, its impact on cognition augments. Aging is known to promote alterations in neuronal structure, loss of synapses, and dysfunction of neuronal networks (37). Also, previous studies have described a decreased global efficiency on PwMS compared to healthy volunteers, suggesting a disrupted topological organization of the WM networks due to impaired structural connections (38). Besides, abnormalities of global efficiency have been associated with negative consequences on cognition impacting different cognitive domains such as memory and attention performance (39–41). As the compensation and adaptation of brain mechanisms probably deteriorate with age and with brain damage, it would appear that brain network dysfunction leads to CI (16). Overall, our results reinforce the protective capacity of CR at any stage of the disease, including in PwMS that suffer cognitive decline.

Our findings entail relevant clinical repercussions as they emphasize the use of the CRQ scale in routine clinical practice to achieve a comprehensive assessment of PwMS and to identify at-risk individuals of cognitive decline. Neurologists should recommend that PwMS participate in early interventions to maximize their brain resources, such as intellectual enhancement or neuropsychological programs.

Our study is not absent of limitations, particularly as our cohort was composed predominantly of relapsing-remitting MS patients, and thus, it limits the capacity to generalize these findings to more advanced phenotypes. However, this is the most common phenotype encountered in the clinic in the current treatment era, with lower rates of worsening and evolution to SPMS in patients compared to earlier natural history cohorts (42). Furthermore, despite the fact that CRQ scores were different in the PwMS-CP and PwMS-CI groups, results remained unchanged when we balanced CRQ scores (data not shown). In addition, CR cannot be measured directly and there is still no consensus as to what is the best proxy for CR (43). Nevertheless, the CRQ measures different intellectual enrichment factors addressing experiences throughout life and is easily applicable in the clinical field due to its brevity and the absence of open responses (22). We do not have longitudinal data on cognitive performance so we were unable to establish a causal effect, yet our results are promising and in accordance with the existing literature. Finally, the inclusion of fMRI in future studies might be useful to further explore compensatory and plasticity mechanisms driven by intellectual enrichment in MS.

In conclusion, CR could have a positive effect on the connectivity of the brain network, which can be observed in patients with more severe clinical impairment. The results presented here highlight the important protective value of CR on cognitive performance, regardless of cognitive status. However, once CI has flourished, over and above the effect of CR, cognition is also influenced by the presence of structural brain damage and aging. This study draws attention to the benefits of promoting an intellectually rich lifestyle in PwMS, as it may have an important impact on their future cognitive status through all stages of the disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee at the Hospital Clinic Barcelona. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.581700/full#supplementary-material

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Performance of Regression-Based Norms for Cognitive Functioning of Persons With Multiple Sclerosis in an Independent Sample

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Background: Cognitive impairment is common in multiple sclerosis (MS). Interpretation of neuropsychological tests requires the use of normative data. Traditionally, normative data have been reported for discrete categories such as age. More recently continuous norms have been developed using multivariable regression equations that account for multiple demographic factors. Regression-based norms have been developed for use in the Canadian population for tests included in the MACFIMS and BICAMS test batteries. Establishing the generalizability of these norms is essential for application in clinical and research settings.

Objectives: We aimed to (i) test the performance of previously published Canadian regression-based norms in an independently collected sample of Canadian healthy controls; (ii) compare the ability of Canadian and non-Canadian regression-based norms to discriminate between healthy controls and persons with MS; and (iii) develop regression-based norms for several cognitive tests drawn from batteries commonly used in MS that incorporated race/ethnicity in addition to age, education, and sex.

Methods: We included 93 adults with MS and 96 healthy adults in this study, with a replication sample of 104 (MS) and 39 (healthy adults). Participants reported their sociodemographic characteristics, and each was administered the oral Symbol Digit Modalities Test (SDMT), the California Verbal Learning Test (CVLT-II), and the Brief Visuospatial Memory Test-Revised (BVMT-R). From the healthy control data, we developed regression-based norms incorporating race, age, education and sex. We then applied existing discrete norms and regression-based norms for the cognitive tests to the healthy controls, and generated z-scores which were compared using Spearman

rank and concordance coefficients. We also used receiver operating characteristic (ROC) curves to compare the ability of each set of norms to discriminate between participants with and without MS. Within the MS samples we compared the ability of each set of norms to discriminate between differing levels of disability and employment status using relative efficiency.

Results: When we applied the published regression norms to our healthy sample, impairment classification rates often differed substantially from expectations (7%), even when the norms were derived from a Canadian (Ontario) population. Most, but not all of the Spearman correlations between z-scores based on different existing published norms for the same cognitive test exceeded 0.90. However, concordance coefficients were often lower. All of the norms for the SDMT reliably discriminated between the MS and healthy control groups. In contrast, none of the norms for the CVLT-II or BVMT-R discriminated between the MS and healthy control groups. Within the MS population, the norms varied in their ability to discriminate between disability levels or employment status; locally developed norms for the SDMT and CVLT-II had the highest relative efficiency.

Conclusion: Our findings emphasize the value of local norms when interpreting the results of cognitive tests and demonstrate the need to consider and assess the performance of regression-based norms developed in other populations when applying them to local populations, even when they are from the same country. Our findings also strongly suggest that the development of regression-based norms should involve larger, more diverse samples to ensure broad generalizability.

Keywords: multiple sclerosis, cognition, regression-based norms, reliability, BICAMS

INTRODUCTION

Over 40% of persons with multiple sclerosis (MS) are thought to experience cognitive impairment which adversely affects social participation, independence, and employment (1, 2). Cognitive impairment at diagnosis has been found to be associated with disability progression over time (3). Neuropsychological assessments objectively evaluate cognitive function, and are increasingly important in the care of persons with MS patients, as new rehabilitative strategies and pharmacologic therapies for cognitive impairments continue to emerge. Given that access to comprehensive neuropsychological assessments is often limited, several abbreviated test batteries have been recommended for use in persons with MS, including the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) (4). Brief Repeatable Battery of Neuropsychological Tests (BRB-N) (5), and the Minimal Assessment of Cognitive Function in MS (MACFIMS) (5, 6). Interpretation of test results for both research and for clinical practice requires the use of normative data, although most available published normative data for these tests were developed in American populations. Application of American norms to Canadian populations is not recommended due to differences in performance between Canadian and American adults on measures of intellectual ability (7). Moreover, published norms were often established in samples that no longer reflect contemporary demographics; for example the proportion of individuals with higher levels of education was lower than in the present day population. Notably, Intelligence Quotient scores have risen over time (8), and use of outdated norms may lead to misclassification of cognitive status by underestimating the normal range of performance (9). In consideration of these issues, recommendations for international validation of the BICAMS were made to encourage its adoption (10).

Traditionally, normative data have been reported for discrete categories, such as age and/or education. More recently, continuous norms have been developed using multivariable regression equations that account for multiple demographic factors simultaneously. Regression-based norms for use in the Canadian population were recently developed for tests included in the MACFIMS battery (11), including the subset of tests included in BICAMS. Because these norms were derived from control populations recruited for other purposes, the number of participants available was fewer than the recommended 100 participants for some tests. In addition, while developed for use in Canada, the controls were drawn from only one region of Canada (i.e., province of Ontario), and the performance of these norms in an independently collected sample of healthy Canadian persons has not yet been assessed. Establishing the generalizability of norms is essential to determine if they may be appropriately applied in clinical and research settings more broadly than those from which the normative samples were drawn.

We sought to (i) test the performance of the previously published Canadian (Ontario) regression-based norms in independently collected samples of healthy controls from other

Canadian regions; (ii) develop local regression-based norms for the tests included in the BICAMS; and (iii) examine differences in impairment classification rates in local healthy controls when applying BICAMS regression-based norms from different populations; and (iv) examine the ability of Canadian and non-Canadian norms to discriminate between local healthy and MS samples.

METHODS

We conducted the primary analysis using MS and healthy control samples from Manitoba, Canada. Manitoba is a central Canadian province with a population of ∼1.4 million people. We replicated our analyses in MS and healthy control samples from the eastern Canadian province of Nova Scotia (population ∼1.0 million), which are described further in the replication section.

Setting and Participants

In Manitoba, we enrolled a subgroup of persons with MS participating in a longitudinal study of immune-mediated inflammatory diseases (the "IMID" study) as previously described (12). Participants were recruited from the single specialized care center for persons with MS in the province. This subgroup of 111 participants attended an IMID study visit between September 2016 and July 2017 which included cognitive testing (13). MS participants were aged ≥18 years, with adequate knowledge of the English language to provide informed consent.

We enrolled healthy controls from September 2018 to September 2019. Inclusion criteria for study participation included aged ≥18 years, with adequate knowledge of the English language to provide informed consent. Exclusion criteria included any chronic medical condition, known cognitive impairment, any positive response to the Structured Clinical Interview for DSM-IV (SCID-IV) screening questions for depressive or anxiety disorders, any head injury associated with loss of consciousness or amnesia, or chronic medication use with the exceptions of contraceptives, hormone replacement therapy, transient antibiotic use, or multivitamins (14). Hypertension, as identified during the study visit (see below), was also an exclusion criterion even if not reported as a diagnosed condition by the participant. We recruited participants using multiple methods including posters placed in hospital, university, and community settings throughout Winnipeg; mail-outs of a study poster to homes in Winnipeg; and word of mouth. Sample size requirements for the development of regression-based norms are 2.5 to 5.5-fold smaller than for the development of discrete norms, while retaining similar or better precision (15), and samples of 100–500 persons are sufficient. Thus, our target sample size was 100.

Participant Characteristics

All participants, including those with MS and healthy participants, underwent standardized assessments and completed questionnaires (12). Participants reported their sociodemographic characteristics including sex, date of birth, ethnicity, years of education, and annual household income as described in detail previously (12). Participants also reported their smoking status; we classified participants who had smoked at least 100 cigarettes as ever smokers (16). We determined body mass index (BMI, kg/m^2) based on height and weight measured at the study visit. Only participants with MS underwent a neurological examination for calculation of the Expanded Disability Status Scale (EDSS) score by an EDSS-certified neurologist.

Neuropsychological Measures

We were primarily interested in the development of local regression-based norms to support an ongoing study examining the influence of vascular and psychiatric comorbidity on cognition in MS (13). The neuropsychological tests conducted examined cognitive domains most often affected in MS, and the comorbidities of interest (17, 18) and included tests of information processing speed, verbal learning and memory, and visual learning and memory. From these tests we examined the test scores comprising the BICAMS, i.e., the oral Symbol Digit Modalities Test (SDMT) (19), the California Verbal Learning Test (CVLT-II; Trial 1–5 total recall score) (20), and the Brief Visuospatial Memory Test-Revised (BVMT-R; summed recall score for all three learning trials) (21). Each participant also completed the Wechsler Test of Adult Reading (WTAR) as an estimate of premorbid IQ.

Analyses

First, we summarized participant characteristics using descriptive characteristics including mean, standard deviation (SD), frequency and percent (%).

Second, to develop regression-based norms in our healthy control group we adapted the approach previously described by Berrigan et al. (22) Specifically, we converted raw scores to scaled scores with a mean of 10 and standard deviation (SD) of 3 based on the cumulative frequency distribution in our control group. Then, we developed a separate regression model for each test or subtest of interest, where the scaled test score was the dependent variable. To account for the bounded distribution of the scaled scores and ensure that predicted values did not fall outside the range of possible values, we used truncated rather than linear regression models. The independent variables were sex (coded as 1 = male, 2 = female), years of education (continuous), age (continuous), age-squared (continuous), and race/ethnicity (coded as 1 = white, 0 = non-white). We included an age-squared term to account for potential non-linear relationships (22). We included race/ethnicity given that cognitive tests may assess individuals of different racial backgrounds differently (23, 24). We did not include estimated pre-morbid IQ as this variable was not included in the development of regression-based norms in MS. For consistency with published Canadian norms, we also report norms without this predictor, and in individuals aged 65 years and under. For each regression model we report the constant and non-standardized coefficients that generate the normative formulae. Model fit was assessed using a pseudo-R^2 calculated as the squared correlation of the observed and predicted values of the dependent variable (25). We assessed

assumptions of homoscedasticity using the White test and residual plots, and assessed assumptions of normality using quantile-quantile plots.

Third, we applied previously published regression-based Canadian norms for the tests where available (11, 22). Two sets of norms were available for the SDMT; we tested both the norms developed using only Ontario participants (11) and the norms developed using participants from Ontario and Nova Scotia (hereinafter Ontario/Nova Scotia) (22). Because these norms were developed in persons aged 18 to 65 years (**Supplementary Table e1**), and accordingly may not perform adequately in older participants, we excluded study participants over age 65 years when examining their performance. Z-scores of ≤-1.5 were classified as impaired. We expected that if the norms performed well, based on a normal distribution ∼7% of our healthy control sample would be classified as impaired on each test.

Fourth, we compared the Canadian regression based norms with non-Canadian regression based norms after applying the norms to generate z-scores. Other norms examined included regression-based norms developed in two other English-speaking populations [Buffalo, New York, United States (hereafter "New York"); Dublin, Ireland (hereafter "Ireland")] (26), the discrete norms available from the published test manuals for each test, and the recently published discrete norms for the SDMT by Strober et al. which were intended to update the previous discrete norms (27). We did not examine regression-based norms for BICAMS developed in non-English-speaking populations (28). The characteristics of the samples used to develop these norms are shown in **Supplementary Table e1**. For these comparisons, we examined the Spearman correlations between the z-scores. We considered correlations of ≤0.39 as low, 0.40–0.59 as moderate, 0.60–0.79 as strong, and ≥0.80 as very strong (29). Because Spearman correlations can establish whether the rank order of participant z-scores are the same, but not whether the same z-score values are assigned, we also examined the concordance coefficients (30). In order to assess the ability of the various norms to differentially discriminate between persons with MS and healthy individuals we compared the area under the receiver operating characteristic (ROC) curve between the various norms, using binary logistic regression, where the dependent variable was MS vs. healthy participant classification.

Given prior reports of an increased frequency of cognitive impairment in persons with MS at greater levels of disability, we examined the ability of each set of norms to discriminate between differing levels of neurologic disability amongst the MS sample (31). We categorized MS participants according to their EDSS scores into mild (0–2.5), moderate (3.0–4.0), and severe (≥4.5) disability groups. We also examined the ability of the norms to discriminate between employed and unemployed persons with MS, where employment status was determined based on the Work Productivity and Impairment Scale (32). Discriminating ability was examined using relative efficiency (RE), where the RE of each set of norms was calculated as the ratio of between group (3 EDSS levels; or 2 employment categories) ANOVA F-statistics. The largest F-statistic represents the greatest discriminative ability.

Replication

Data from an independent sample of MS participants and healthy controls, collected in Nova Scotia, Canada, were used to repeat the analyses comparing Canadian and non-Canadian regression-based norms, including correlations between the norms and their ability to discriminate between healthy and MS samples. These participants were enrolled in an ongoing longitudinal study of attention network functioning in MS and were recruited from the single specialized MS care center in that province. Unlike the Manitoba sample, these MS participants were selected to have an EDSS <4.5, with an age range from 20 to 60 years old. Exclusion criteria included insufficient visual acuity or impaired dexterity that would impede performance on cognitive tasks) or comorbid conditions that were likely to have a significant impact on their cognition (e.g., neurologic disorders other than MS, diagnosed learning disability, previous head injury with loss of consciousness, and sub-optimally managed psychiatric disorder as determined by clinic staff). As the independent Nova Scotia sample was selected to have no more than moderate levels of neurologic disability, only one participant fell within the "severe" EDSS category of >4.5 used in the previous analyses. Therefore, these participants were instead divided into only two categories: mild (0–2.5) and moderate (3.0–4.5). The data of 104 MS participants, tested between August 2016 and July 2018, were used in the current study replication. Healthy control participants ($n = 39$) recruited over this time period met the same exclusion criteria as the MS group but had no history or family history of MS and no history of psychiatric disorder; they were matched to the MS group based on age, years of education, and sex. Although all necessary cognitive measures were available in this dataset, several demographic variables were not collected: Ethnicity, annual household income, smoking status, and body mass index.

Statistical analyses were conducted using SAS V9.4 (SAS Institute Inc., Cary, NC) and SPSS Version 25 (IBM Corp., Armonk, NY).

RESULTS

Throughout, we present the findings in Manitoba followed by the findings in the Nova Scotia replication sample. Of the 103 healthy participants from Manitoba, 96 were under age 65 years, and of 111 participants with MS, 93 were under age 65 years. The healthy participants were younger on average, but the age range of the healthy participants (18.2–64.4) was similar to that of the participants with MS (20.8–63.8) years. Most participants in each group were women, although the proportion who were women was higher in the MS group (**Table 1**). The average number of years of education was consistent with at least some post-secondary education in both groups although the healthy control group averaged 2.4 more years of education than the MS group.

TABLE 1 | Characteristics of participants.

Characteristic **Manitoba**	**Healthy (all)**	**Healthy ≤65 years**	**MS**	**Std Diff[a]**	***P*-value[a]**
N	103	96	93		
Age (year), mean (SD)	38.7 (16.3)	36.1 (13.6)	45.6 (9.6)	0.81	<0.0001
Women, *n* (%)	68 (66.0)	64 (66.7)	77 (82.8)	0.16	0.011
White, *n* (%)	85 (82.5)	79 (82.3)	74 (80.4)	0.02	0.74
Years of education, mean (SD)	16.7 (3.0)	16.6 (3.0)	14.2 (2.6)	0.85	<0.0001
Annual income, *n* (%)					0.48
<$50,000	33 (32.0)	32 (33.3)	26 (28.0)	0.053	
≥$50,000	60 (58.3)	55 (57.3)	61 (65.6)	0.083	
I do not wish to answer	10 (9.7)	9 (9.4)	6 (6.4)	0.03	
Employed[b], *n* (%)	82 (79.6)	81 (81.4)	54 (58.7)	0.23	<0.0001
Ever Smoker, *n* (%)	13 (12.6)	12 (18.2)	54 (58.1)	0.40	<0.0001
BMI (kg/m^2), mean (SD)	25.5 (4.7)	25.4 (4.7)	29.0 (6.6)	0.63	<0.0001
FSIQ, mean (SD)	110 (7.8)	109.9 (7.7)	106.3 (8.2)	0.45	0.0022
Nova Scotia	**Healthy**		**MS**	**Std Diff[c]**	***P*-value[c]**
N	39		104		
Age (year), mean (SD)[d]	49.4 (9.7)		47.0 (8.6)	0.27	0.19
Women, *n* (%)	35 (89.7)		91 (87.5)	0.07	0.71
Years of education, mean (SD)	15.1 (1.5)		14.6 (1.8)	0.29	0.11

[a]For comparison of healthy (n = 96) and MS (n = 93) participants aged 65 years and under in Manitoba; [b]missing for one person with MS; [c]For comparison of healthy (n = 39) and MS (n = 104) participants in Nova Scotia; [d]all healthy participants <age 60 years.

TABLE 2 | Raw score to scaled score conversions.

Scaled Score	**SDMT**	**CVLT-II verbal learning**	**BVMT-R total recall**
2	<40	<30	<10
3	40–43	30–31	10–11
4	44–46	32–38	12
5	47–50	39–40	13–14
6	51–54	41–43	15–19
7	55–57	45–47	20–22
8	58–59	48–49	23–24
9	60–62	50–55	25–26
10	63–65	56–57	27–28
11	66–69	58–61	29–30
12	70–73	62–63	31–32
13	74–78	64	33
14	79–80	65–68	34
15	81	69	35
16	82–83	70	36
17	≥84	>70	
18			

Race/ethnicity did not differ between the two groups, nor did estimated household income.

In the replication sample, most participants were also women, and the average number of years of education was consistent with at least some post-secondary education (**Table 1**).

Impairment Classification Rates

Table 2 shows raw score to scaled score conversions used to develop the regression-based norms in healthy controls aged 65 years and younger in Manitoba. **Table 3** shows the regression-based formulae with and without race as a covariate. The degree of variance in the cognitive tests explained by demographic factors varied slightly between tests.

When we applied the published regression norms to the healthy Manitoba sample, the impairment classification rates often differed substantially from the expected rate of 7%, even when the norms were derived from another Canadian (Ontario) population. The exceptions for the SDMT were the regression-based norms from Ontario/Nova Scotia and New York; and for the CVLT were the regression-based norms from New York, and the discrete norms (**Figure 1A**).

When the published regression norms and locally developed Manitoba norms were applied to the independent Nova Scotia healthy sample, impairment classification rates were lower and more often within the expected range based on a normal population distribution (i.e., 7%) (**Figure 1B**). However, there were notable outliers: 30.8% and 28.2% of controls in the replication sample of healthy controls were impaired on the CVLT-II and BVMT-R, respectively, using the New York norms; 25.6% were impaired on BVMT-R using the Ontario norms; and 25.6% impaired on the BVMT-R using the discrete norms.

Correlations and Concordance Between Norms

In the Manitoba sample, most, but not all of the Spearman correlations between z-scores based on existing published norms

TABLE 3 | Regression-based norms with and without incorporating race as a demographic predictor derived from healthy controls aged ≤65 years[a].

Test	Constant	Std Err	Sex	Age	Age^2	Educ	Race	Pseudo-R^2
SDMT	8.16	2.62	0.74, $p = 0.20$	−0.088, $p = 0.0005$	0.002, $p = 0.19$	0.005, $p = 0.96$		0.15
SDMT	6.76	2.52	0.98, $p = 0.08$	−0.080, $p = 0.0012$	0.002, $p = 0.21$	−0.024, $p = 0.80$	1.84, $p = 0.0082$	0.21
CVLT-II, verbal learning	3.81	2.71	1.96, $p = 0.0009$	−0.024, $p = 0.36$	0.002, $P = 0.39$	0.15, $P = 0.13$		0.13
CVLT-II, verbal learning	2.99	2.68	2.11, $p = 0.0004$	−0.019, $p = 0.46$	0.002, $p = 0.42$	0.14, $p = 0.18$	1.09, $p = 0.14$	0.15
BVMT-R, total recall	8.45	2.53	1.02, $p = 0.066$	−0.084, $p = 0.0007$	0.001, $p = 0.45$	−0.028, $p = 0.77$		0.18
BVMT-R, total recall	7.51	2.49	1.18, $p = 0.032$	−0.078, $p = 0.0014$	0.001, $p = 0.48$	−0.048, $p = 0.61$	1.24, $p = 0.069$	0.21

[a] *Truncated regression; Sex 1 = male, 2 = female; age in years and centered at 36.12; education in years; SDMT, Symbol Digit Modalities Test; CVLT-II, California Verbal Learning Test; BVMTR, Brief Visual Memory Test-Revised.*

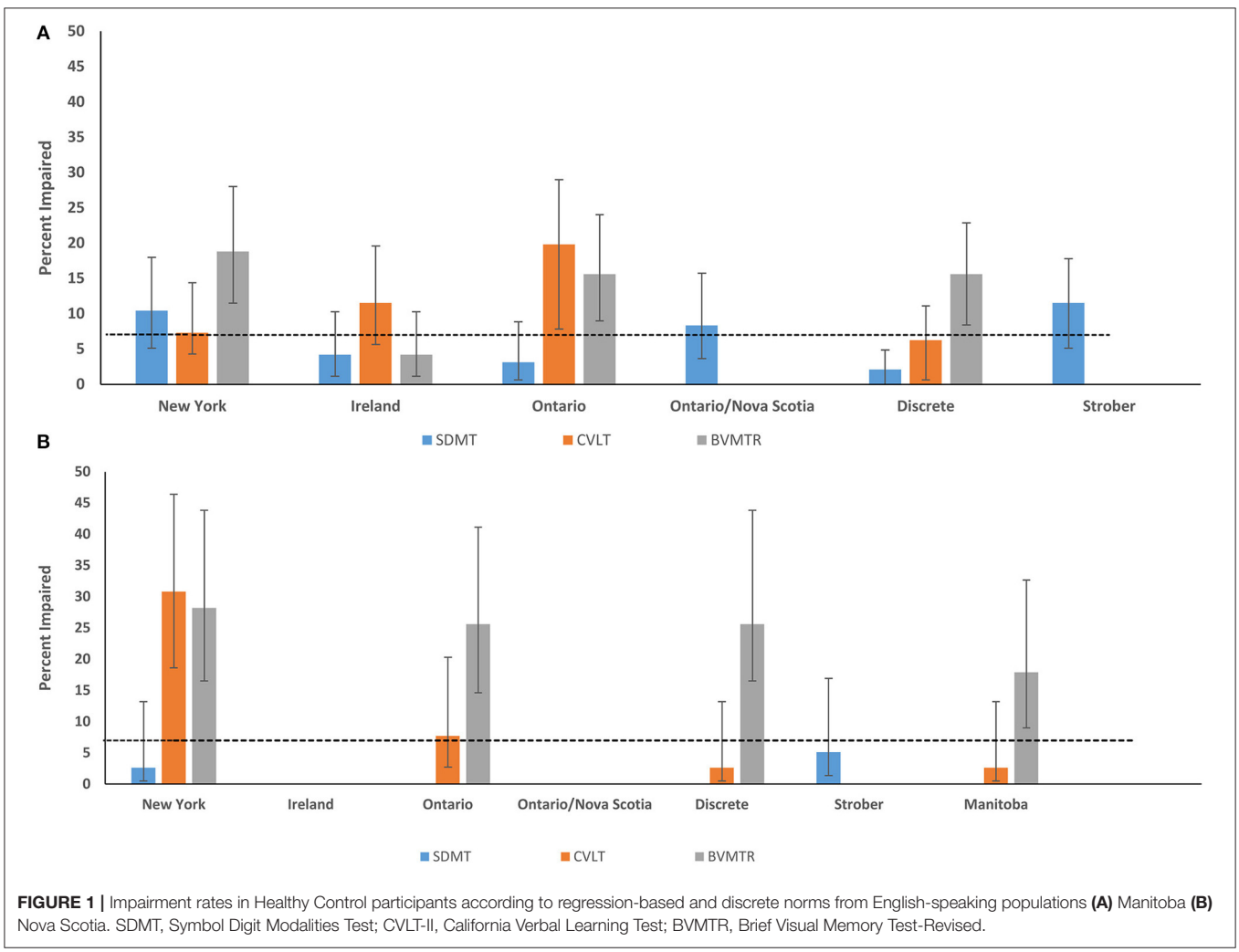

FIGURE 1 | Impairment rates in Healthy Control participants according to regression-based and discrete norms from English-speaking populations **(A)** Manitoba **(B)** Nova Scotia. SDMT, Symbol Digit Modalities Test; CVLT-II, California Verbal Learning Test; BVMTR, Brief Visual Memory Test-Revised.

for the same cognitive test exceeded 0.90 (**Table 4**). However, concordance coefficients were often lower, ranging from 0.45 to 0.96 (**Table 4**). The discrepancies between norms appeared to be greatest between the norms from Ireland as compared to all other norms. This pattern of high correlation coefficients, with the greatest discrepancies between the norms from Ireland and other

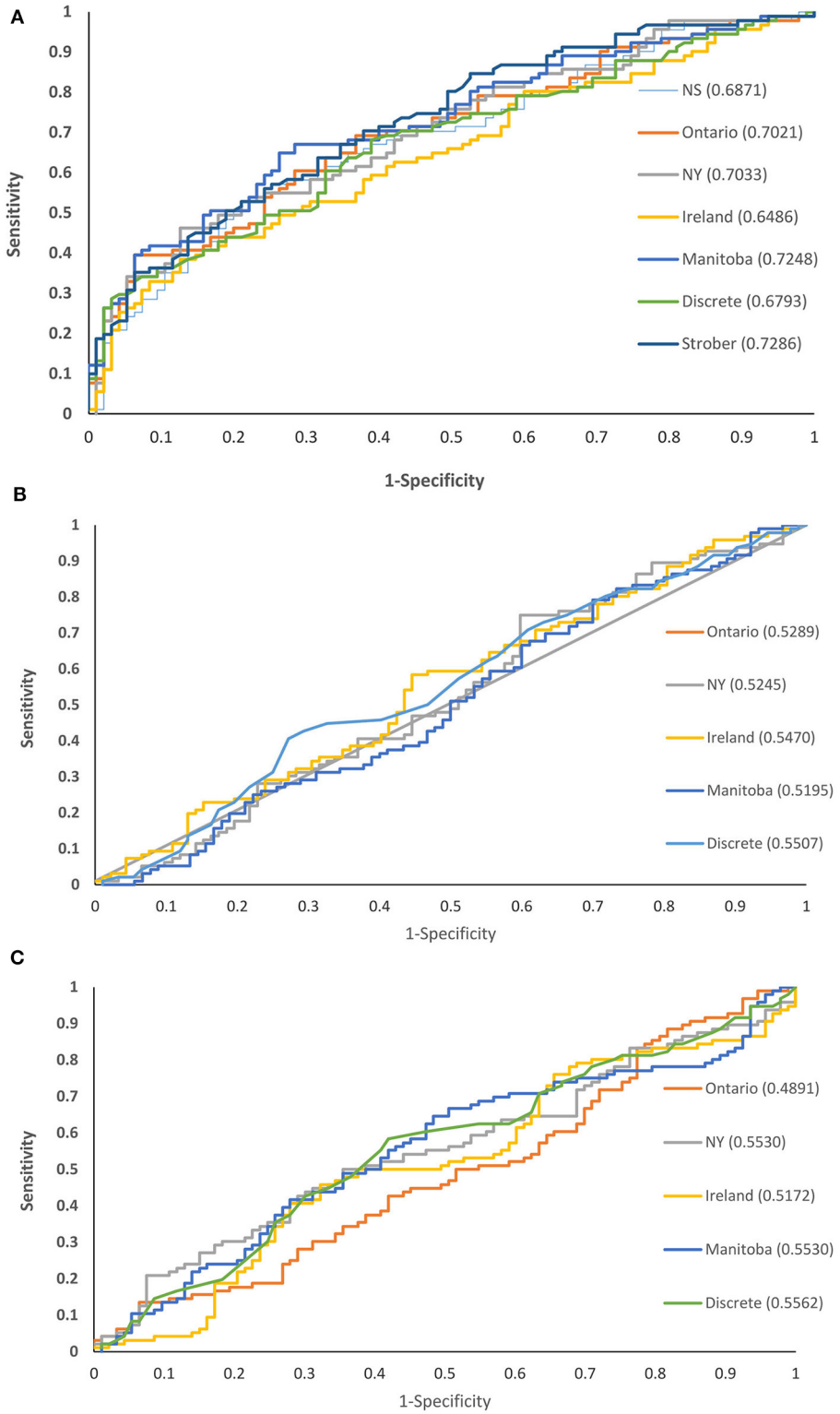

FIGURE 2 | Receiver operating characteristic curves for cognitive test norms comparing persons with and without multiple sclerosis in Manitoba: **(A)** SDMT **(B)** CVLT-II **(C)** BVMT-R.

norms, was replicated in the independent Nova Scotia sample (**Supplementary Table e2**). In addition, correlations between the locally developed Manitoba norms and all other norms showed the same pattern.

Ability of BICAMS Norms to Discriminate Between MS and Healthy Control Groups

All of the norms for the SDMT discriminated between the MS and healthy control groups, based on ROC analyses, but they differed in their ability to do so (**Figure 2A**). The area under the ROC curve (AUC) was highest for the Strober et al. discrete norms and the locally developed Manitoba norms (without race for comparability), and the AUC was lowest for the Irish norms. As compared to the Manitoba norms (AUC 0.72; 95% CI: 0.65–0.80), the Strober (AUC 0.73; 95% CI: 0.66–0.80, $p = 0.81$) and New York norms (AUC 0.70; 95% CI: 0.63–0.78, $p = 0.18$) did not differ. As compared to the Manitoba norms, the Ontario (AUC 0.70; 95% CI: 0.63–0.78, $p = 0.01$), Ontario/Nova Scotia (AUC 0.69; 95% CI: 0.61–0.76, $p = 0.0038$), Irish (AUC 0.65; 95% CI: 0.57–0.73, $p = 0.0002$) and discrete norms from the SDMT manual (AUC 0.68; 95% CI: 0.60–0.76, $p < 0.0001$) did not discriminate as well.

None of the norms for the CVLT-II verbal learning discriminated between the MS and healthy control groups (**Figure 2B**). The discriminating ability of the Manitoba norms (AUC 0.50; 95% CI: 0.42–0.59) did not differ from that of the Ontario (AUC 0.53; 95% CI: 0.45–0.62, $p = 0.68$), New York (AUC 0.52; 95% CI: 0.44–0.61, $p = 0.32$), Irish (AUC 0.55; 95% CI: 0.63–0.63, $p = 0.52$) or discrete (AUC 0.55; 95% CI: 0.47–0.63, $p = 0.057$) norms.

None of the norms for the BVMT-R total recall discriminated between the MS and healthy control groups (**Figure 2C**). The discriminating ability of the Manitoba norms (AUC 0.55; 95% CI: 0.47–0.64) did not differ from that of the Ontario (AUC 0.49; 95% CI: 0.41–0.57, $p = 0.44$), New York (AUC 0.55; 95% CI: 0.47–0.64, $p = 1.0$), Irish (AUC 0.52; 95% CI 0.43–0.60, $p = 0.083$) or discrete (AUC 0.56; 95% CI: 0.47–0.64, $p = 0.78$) norms.

Similarly, based on ROC analyses of the independent Nova Scotia sample, all norms for the SDMT discriminated between the MS and healthy control groups, while none of the norms for the BVMT-R total recall discriminated between groups (**Supplementary Figure e1**). However, unlike the Manitoba sample, all norms for the CVLT-II verbal learning did discriminate between MS and healthy control groups.

Ability of Different Norms to Discriminate Between MS Participants With Differing Levels of Disability or Employment Status

We next examined whether application of the various norms influenced the extent to which the tests discriminated between differing levels of disability based on the EDSS, amongst individuals within the Manitoba MS cohort. For the SDMT, the Manitoba norms were best able to discriminate between disability groups (**Table 5**). The relative efficiency (RE) for the Ontario and Strober norms exceeded 0.92 compared to the Manitoba norms but the remaining norms had substantially lower RE of 0.52–0.54. For the CVLT-II verbal learning, the Manitoba norms were again best able to discriminate between disability groups. The New York norms had a similar discriminating ability with a RE of 0.97. The remaining norms had lower RE of 0.36–0.69. For the BVMT-R total recall, the discrete norms had the best discriminating ability, while the New York norms had the lowest RE. Considering only the Manitoba norms, the BVMT-R best discriminated between differing disability levels, followed by the SDMT and CVLT-II. This same pattern was seen for the Ontario, Ireland and discrete norms from the manual, but not for the New York norms where the BVMT-R had the poorest discriminating ability.

Similar to the findings for disability, the various norms differed in their ability to discriminate between employed and unemployed participants with MS. For the SDMT, the Manitoba norms best discriminated between employed and unemployed participants. For the CVLT-II verbal learning, the Ontario norms were the best discriminator, followed closely by the Manitoba norms which were similar with a RE of 0.95. For the BVMT-R, the discrete norms from the manual discriminated best between employed and unemployed participants. Considering only the Manitoba norms, the BVMT-R discriminated better than the SDMT, followed by the CVLT-II. This pattern was consistent for the Ontario, Ireland, and discrete norms from the manual, but not for the New York norms where the BVMT-R had the poorest discriminating ability.

In the sample of 104 MS participants from Nova Scotia, for the SDMT, the Ireland norms were best able to discriminate between the two (i.e., mild vs. moderate) disability groups (**Table 5**). The New York and Ontario/Nova Scotia norms had the next highest RE at 0.83 and 0.81, respectively. Regardless of the norms used, the CVLT-II verbal learning and BVMT-R total recall were unable to discriminate between mild vs. moderate disability groups. The Nova Scotia replication sample did not collect data regarding employment.

DISCUSSION

In this cross-sectional study, we applied a set of previously developed regression-based norms from Ontario, Canada for tests comprising the BICAMS, to an independently collected healthy sample from Manitoba, Canada to assess their generalizability. We also replicated our findings in a second, smaller, normative sample from Nova Scotia, Canada. In healthy controls, the rates of impairment differed from standard population expectations, sometimes being higher than expected and sometimes being lower. The application of regression-based norms developed in other non-Canadian English-speaking populations also produced variable impairment rates that differed from expectations, as did the discrete norms from the test manuals. All of the norms differed in their ability to discriminate between MS and healthy populations from Manitoba, and between Manitobans with MS who had differing levels of disability or employment status. The local Manitoba norms generally had better discriminating ability in the Manitoba sample than other norms, but the CVLT-II and BVMT-R were

TABLE 4 | Spearman two-tailed correlation coefficients and concordance coefficients for the association between different norms in Manitoba.

	Healthy controls ($n = 96$)		Multiple sclerosis ($n = 93$)	
	Correlation coefficient (95% CI)	Concordance coefficient (95% CI)	Correlation coefficient (95% CI)	Concordance coefficient (95% CI)
SDMT				
$SDMT_{NY}$-$SDMT_{IRE}$	0.88 (0.83, 0.92)	0.66 (0.57, 0.74)	0.94 (0.91, 0.96)	0.70 (0.62, 0.77)
$SDMT_{NY}$-$SDMT_{ONT}$	0.96 (0.94, 0.98)	0.86 (0.90, 0.93)	0.96 (0.94, 0.97)	0.91 (0.87, 0.94)
$SDMT_{NY}$-$SDMT_{ONT/NS}$	0.98 (0.97, 0.99)	0.96 (0.95, 0.98)	0.97 (0.96, 0.98)	0.93 (0.90, 0.95)
$SDMT_{IRE}$-$SDMT_{ONT}$	0.86 (0.80, 0.91)	0.73 (0.65, 0.80)	0.93 (0.90, 0.95)	0.81 (0.74, 0.86)
$SDMT_{IRE}$-$SDMT_{ONT/NS}$	0.89 (0.84, 0.93)	0.65 (0.74, 0.80)	0.91 (0.87, 0.94)	0.76 (0.68, 0.82)
$SDMT_{ONT}$-$SDMT_{ONT/NS}$	0.97 (0.96, 0.98)	0.95 (0.93, 0.97)	0.97 (0.95, 0.98)	0.96 (0.94, 0.97)
$SDMT_{NY}$-$SDMT_{DISCRETE}$	0.91 (0.87, 0.94)	0.83 (0.77, 0.88)	0.93 (0.90, 0.95)	0.83 (0.77, 0.88)
$SDMT_{NY}$-$SDMT_{STROBER}$	0.92 (0.89, 0.95)	0.86 (0.81, 0.90)	0.92 (0.88, 0.95)	0.90 (0.86, 0.94)
$SDMT_{IRE}$-$SDMT_{DISCRETE}$	0.87 (0.81, 091)	0.72 (0.63, 0.78)	0.92 (0.88, 0.95)	0.82 (0.76, 0.87)
$SDMT_{IRE}$-$SDMT_{STROBER}$	0.76 (0.65, 0.83)	0.69 (0.83, 0.78)	0.87 (0.81, 0.92)	0.74 (0.65, 0.82)
$SDMT_{ONT}$-$SDMT_{DISCRETE}$	0.96 (0.94, 0.97)	0.96 (0.93, 0.97)	0.97 (0.96, 0.98)	0.96 (0.94, 0.97)
$SDMT_{ONT}$-$SDMT_{STROBER}$	0.92 (0.88, 0.95)	0.89 (0.84, 0.92)	0.95 (0.91, 0.96)	0.92 (0.88, 0.95)
$SDMT_{NS}$-$SDMT_{DISCRETE}$	0.93 (0.90, 0.96)	0.90 (0.86, 0.93)	0.94 (0.90, 0.96)	0.92 (0.88, 0.94)
$SDMT_{DISCRETE}$-$SDMT_{STROBER}$	0.93 (0.89, 0.95)	0.91 (0.87, 0.94)	0.92 (0.87, 0.95)	0.90 (0.86, 0.93)
CVLT				
$CVLT_{NY}$-$CVLT_{IRE}$	0.74 (0.64, 0.82)	0.68 (0.57, 0.76)	0.83 (0.76, 0.89)	0.73 (0.64, 0.80)
$CVLT_{NY}$-$CVLT_{ONT}$	0.87 (0.82, 0.91)	0.79 (0.71, 0.85)	0.94 (0.91, 0.96)	0.92 (0.88, 0.94)
$CVLT_{IRE}$-$CVLT_{ONT}$	0.94 (0.91, 0.96)	0.80 (0.74, 0.85)	0.94 (0.91, 0.96)	0.80 (0.74, 0.85)
$CVLT_{NY}$-$CVLT_{DISCRETE}$	0.93 (0.89, 0.95)	0.83 (0.77, 0.88)	0.96 (0.94, 0.97)	0.90 (0.86, 0.93)
$CVLT_{IRE}$-$CVLT_{DISCRETE}$	0.86 (0.79, 0.72)	0.80 (0.72, 0.85)	0.89 (0.82, 0.92)	0.84 (0.78, 0.88)
$CVLT_{ONT}$-$CVLT_{DISCRETE}$	0.90 (0.85, 0.93)	0.65 (0.57, 0.73)	0.94 (0.91, 0.96)	0.82 (0.76, 0.87)
BVMTR				
$BVMTR_{NY}$-$BVMTR_{IRE}$	0.85 (0.79, 0.90)	0.45 (0.36, 0.53)	0.87 (0.81, 0.91)	0.36 (0.28, 0.43)
$BVMTR_{NY}$-$BVMTR_{ONT}$	0.90 (0.85, 0.93)	0.85 (0.80, 0.89)	0.92 (0.88, 0.95)	0.85 (0.79, 0.89)
$BVMTR_{IRE}$-$BVMTR_{ONT}$	0.97 (0.96, 0.98)	0.51 (0.43, 0.58)	0.96 (0.95, 0.98)	0.49 (0.41, 0.56)
$BVMTR_{NY}$-$BVMTR_{DISCRETE}$	0.85 (0.78. 0.90)	0.66 (0.75, 0.81)	0.88 (0.82, 0.92)	0.75 (0.67, 0.82)
$BVMTR_{IRE}$-$BVMTR_{DISCRETE}$	0.97 (0.95, 0.98)	0.68 (0.60, 0.74)	0.96 (0.93, 0.97)	0.59 (0.51, 0.66)
$BVMTR_{ONT}$-$BVMTR_{DISCRETE}$	0.98 (0.97, 0.99)	0.91 (0.88, 0.94)	0.99 (0.98, 0.99)	0.96 (0.94, 0.97)

SDMT, Symbol Digit Modalities Test; CVLT-II, California Verbal Learning Test; BVMTR, Brief Visual Memory Test-Revised; ONT, Ontario; NY, New York; NS, Nova Scotia; IRE, Ireland.

still poor at discriminating between healthy participants and participants with MS. A prior report in a Belgian sample also found that the CVLT-II did not discriminate between persons with and without MS (33). Prior studies examining the sensitivity of neuropsychological tests suggest that the SDMT discriminates best between people with and without MS (34), and the SDMT is commonly found to be the test most associated with other clinically relevant factors (3). This high sensitivity of the SDMT to cognitive impairment in MS has been attributed to its assessment of commonly affected cognitive abilities including processing speed and working memory, as well as its requirements for efficient visual scanning and oculomotor functioning (27). Overall, our findings indicate that using regional norms to interpret all BICAMS tasks is likely to be most informative.

Spearman correlations between the different norms all exceeded 0.75 and most correlations exceeded 0.90. However, concordance coefficients were lower, indicating that while the norms rank ordered participants similarly, the absolute z-scores differed. Notably, in the Manitoba and Nova Scotia samples, concordance was lowest between the norms from Ireland and the other English language norms, which were developed in regions of Canada or the United States; potentially reflecting greater cultural differences between Ireland and North America than among North American regions for this verbal memory test. A prior study found that nationality influences performance on all three BICAMS tests, even after adjusting for age and years of education (35). That study highlighted the importance of considering both the language and culture of the individual being tested and called for additional studies across countries with common languages to address the potential influences of cultural factors. An approach by which BICAMS can be validated in other languages has been recommended (10) and a systematic review in 2018 reported on the performance of BICAMS as translated from English into 11 languages, following

TABLE 5 | Ability of various norms to discriminate differing levels of disability and employment status among participants with MS.

		Manitoba sample ($N = 93$)					
		Disability			Employment		
Test	**Norm**	***F*-test**	***P*-value**	**Relative efficiency**	***F*-test**	***P*-value**	**Relative efficiency**
SDMT	Manitoba	10.2	0.0001	1	5.19	**0.025**	1
	Ontario	9.38	0.0002	0.92	4.42	**0.038**	0.85
	Ontario/Nova Scotia	5.46	0.0058	0.54	1.90	0.17	0.37
	Strober	8.07	0.0006	0.79	2.98	0.088	0.57
	Discrete	7.82	0.0007	0.54	4.19	**0.044**	0.81
	New York	5.3	0.0067	0.52	2.91	0.092	0.56
	Ireland	5.21	0.0072	0.51	4.64	**0.034**	0.89
CVLT-II	Manitoba	8.45	0.0004	1	3.72	0.057	0.95
	Ontario	5.87	0.004	0.69	3.90	0.051	1
	Discrete	5.49	0.0056	0.65	3.13	0.080	0.80
	New York	8.16	0.0006	0.97	2.69	0.10	0.69
	Ireland	3.08	0.051	0.36	3.05	0.084	0.78
BVMT-R	Manitoba	12.99	<0.0001	0.79	6.92	**0.01**	0.60
	Ontario	14.12	0.0001	0.86	8.16	**0.0053**	0.71
	Discrete	16.45	<0.00001	1	11.54	**0.001**	1
	New York	6.38	0.0026	0.39	1.92	0.17	0.17
	Ireland	13	<0.0001	0.86	9.92	**0.0022**	0.86
		Nova Scotia Sample (N = 104)					
		Disability[a]					
SDMT	Manitoba	15.032	<0.0001	0.79			
	Ontario	13.126	<0.0001	0.69			
	Ontario/Nova Scotia	15.395	<0.0001	0.81			
	Strober	11.026	0.001	0.58			
	Discrete	14.052	<0.0001	0.74			
	New York	15.754	<0.0001	0.83			
	Ireland	18.916	<0.0001	1			
CVLT-II	Manitoba	2.119	0.149	0.54			
	Ontario	3.914	0.051	1			
	Discrete	2.329	0.130	0.60			
	New York	2.685	0.104	0.68			
	Ireland	3.302	0.072	0.84			
BVMT-R	Manitoba	1.246	0.267	0.77			
	Ontario	1.608	0.208	1			
	Discrete	1.449	0.231	0.90			
	New York	0.984	0.324	0.61			
	Ireland	0.977	0.325	0.61			

SDMT, Symbol Digit Modalities Test; CVLT-II, California Verbal Learning Test; BVMTR, Brief Visual Memory Test-Revised; [a]For these analyses disability was divided into two groups instead of three: "mild" (EDSS 0–2.5) and "moderate" (EDSS 3.0–4.5), as only 1 participant would have fallen into the category of "severe" (EDSS of 4.5 and above) used in Manitoba. Employment status not available for Nova Scotia sample. Bold indicates statistical significance.

which performance was assessed (28). However, within countries, including Canada, where inhabitants may use one or more languages and/or are members of different cultural groups, there may be a need for particular effort to ensure appropriate norms are applied.

In principle, clinicians, and researchers may choose to use discrete norms that are commercially available for the cognitive tests they employ, locally validated norms, or regression-based norms from other populations. For example, regression-based norms derived from a Canadian sample have been employed in Sweden, albeit modified to exclude educational level (36). A large multi-center trial of exercise and cognitive rehabilitation will be applying Dutch norms at the Denmark site (37). Notably, even when we employed only norms developed in

other regions of Canada, our local norms, and discrete norms from the manuals for each test that are used in clinical practice, we observed meaningful variations in impairment classification rates and in the ability to discriminate between and within groups. This reflects the differences in the absolute z-scores, as demonstrated by the lower concordance coefficients than correlation coefficients. These differences may reflect differences in the healthy populations enrolled, as well as differences in the approaches used to develop the norms. For example, Walker *et al.* used raw test scores in their regression models and did not incorporate a non-linear term for age (11), while Berrigan et al. used scaled scores and incorporated a non-linear term for age that reflected non-linear findings reported in large samples (22). Our findings suggest that methodological issues such as these constitute an important component of the wide variation in the frequency of cognitive impairment reported in the MS literature [reviewed in Chiaravalloti and DeLuca (38)]. Differences in the ability to discriminate between healthy and MS groups, and between groups of persons with MS at differing levels of neurologic disability and employment status, also highlight how the use of different norms affects the identification of factors influencing cognitive outcomes.

Within the Manitoba healthy sample, the contribution of demographic characteristics to cognitive performance also varied across the three cognitive tests evaluated, with the variance explained ranging from 15 to 21%, consistent with prior reports (26). The poorer performance seen on the SDMT and BVMT-R with increasing age is consistent with prior reports in healthy populations (39, 40). Sex was associated with performance of the CVLT-II, but not the SDMT or BVMT-R. One prior report suggested that the association of sex SDMT performance is only seen for the written version of this test, with women having better scores than men, whereas this is not the case of for the oral version used here and recommended for persons with MS (39). Education was not associated with cognitive performance, but most of our healthy sample was well-educated. Race predominantly contributed to performance on the SDMT in our sample although the association between race, ethnicity and performance of cognitive tests is well-recognized (40).

Raw scores on cognitive tests have been demonstrated to have higher sensitivity than demographically-corrected scores for discriminating between persons with and without cognitive impairment, but demographically-corrected scores have higher specificity (41). Several options exist for demographically correcting scores. Discrete norms are easy to develop but require continuous variables such as age to be categorized. This creates somewhat arbitrary and discontinuous changes in expected performance for individuals at the boundaries of those categories and relatively large sample sizes are required to develop precise norms with smaller categories that address this issue (15). Regression-based norms have become popular because they do not categorize continuous variables, and the improved efficiency of estimation allows for the use of substantially smaller sample sizes while providing more precise estimates. For the BICAMS, the international validation standards recommend that the minimum sample size is 65 healthy volunteers, provided that they are group matched on demographics to an MS sample (10). Samples of $\geq$150 persons or more are encouraged for generalizability. We used linear regression models to develop our norms as is common in the literature. This approach is affected by whether model assumptions are met, and model assumptions were met in this study. Nonetheless, skewness may interfere with norm accuracy (42), and outliers may exert a substantial influence on the norms that are developed, particularly in smaller samples. Linear regression examines the relationship between the conditional mean of the dependent variable to the independent variables of interest, and assumes that this adequately represents relationships across the entire distribution of the dependent variable. Moreover, traditional linear regression does not account for the fact that cognitive tests typically have a limited range of scores and therefore, we employed a truncated regression model to account for this issue.

Limitations of this study should be recognized. To ensure comparability with existing Canadian-Ontario regression-based norms, we did not include participants over age 65 years. However, after restricting our analyses to persons who were aged $\leq$65 years, we had 96 participants for developing local norms in Manitoba. While this exceeds the minimum 65 persons recommended in the BICAMS international standards for validation (10), it is slightly <100 recommended based on simulation studies (15). Like the healthy samples used to develop regression-based norms for BICAMS that we evaluated here (**Supplementary Table e1**), our healthy sample predominantly included women ($n = 32$ men). Most of our study population were white, thus further work is needed to develop norms that account for the racial/ethnic diversity in Canada and elsewhere. This is particularly important as recognition grows of the burden of MS in populations traditionally considered to be at a lower risk of MS such as indigenous Canadians and African Americans (43, 44). We did not capture acculturation which may also influence performance of norms (45). On average, the healthy control sample in Manitoba was younger than the MS sample, and more highly educated; differences in sex distribution were more modest as indicated by the standardized difference of <0.20. Norms should be applied cautiously in populations with different characteristics than those in whom they were developed due to limitations in generalizability, as illustrated by our findings. However, while the samples differed on average, the age and years of education distributions overlapped.

Regression-based norms have advantages over discrete norms. However, our findings emphasize the value of local norms when interpreting the findings of cognitive tests (46) and demonstrate the need to consider and assess the performance of regression-based norms developed in other populations when applying them to local populations, even when they are from the same country. This is important to avoid misclassifying individuals as to whether they are cognitively impaired or unimpaired. Our

findings also strongly suggest that the development of regression-based norms should involve larger, more diverse samples to ensure broad generalizability. Specifically, greater representation is needed of men, individuals over age 65 years, and of varying racial, ethnic, and social backgrounds.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because some participants did not agree to data sharing; some data may be accessible to qualified investigators with the appropriate ethical approvals and data use agreements. Requests to access the datasets should be directed to Ruth Ann Marrie, rmarrie@hsc.mb.ca.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Manitoba Health Research Ethics Board and the Nova Scotia Health Authority Research Ethics Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RM, JF, and RP conceived of the idea. RM, RP, CF, JK JB, LG, EM, JM, CB, and JF obtained study funding. RM and CW conducted the statistical analyses and drafted the manuscript. RM, RP, CW, CF, JK, JB, LG, EM, JM, CB, and JF revised the manuscript and approved of the final version. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.621010/full#supplementary-material

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Cognitive Processes Underlying Verbal Fluency in Multiple Sclerosis

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Background: Verbal fluency (VF) has been associated with several cognitive functions, but the cognitive processes underlying verbal fluency deficits in Multiple Sclerosis (MS) are controversial. Further knowledge about VF could be useful in clinical practice, because these tasks are brief, applicable, and reliable in MS patients. In this study, we aimed to evaluate the cognitive processes related to VF and to develop machine-learning algorithms to predict those patients with cognitive deficits using only VF-derived scores.

Methods: Two hundred participants with MS were enrolled and examined using a comprehensive neuropsychological battery, including semantic and phonemic fluencies. Automatic linear modeling was used to identify the neuropsychological test predictors of VF scores. Furthermore, machine-learning algorithms (support vector machines, random forest) were developed to predict those patients with cognitive deficits using only VF-derived scores.

Results: Neuropsychological tests associated with attention-executive functioning, memory, and language were the main predictors of the different fluency scores. However, the importance of memory was greater in semantic fluency and clustering scores, and executive functioning in phonemic fluency and switching. Machine learning algorithms predicted general cognitive impairment and executive dysfunction, with F1-scores over 67–71%.

Conclusions: VF was influenced by many other cognitive processes, mainly including attention-executive functioning, episodic memory, and language. Semantic fluency and clustering were more explained by memory function, while phonemic fluency and switching were more related to executive functioning. Our study supports that the multiple cognitive components underlying VF tasks in MS could serve for screening purposes and the detection of executive dysfunction.

Keywords: multiple sclerosis, cognitive, neuropsychology, fluency, processing speed, machine learning

INTRODUCTION

Multiple sclerosis (MS) is a demyelinating disease and the most common cause of non-traumatic disability in working-age adults (1). It presents different lesions and cortical/ subcortical gray matter brain damage, as well as functional disconnection (2). The most prominent cognitive symptoms are slowed cognitive processing speed, attention, episodic memory, and executive function impairments, including verbal fluency (VF) deficits, and visuospatial analysis impairment (3).

Executive functions are an essential part of cognitive assessment and include different specialized cognitive processes. One of these cognitive processes is fluency, understood as the ability to generate non-overlearned responses after a cue presentation in a certain time window (4). In this regard, verbal fluency tasks are some of the most widely used tasks, and according to the cue presentation, it is possible to distinguish two modalities: words of a specific semantic field, called semantic fluency; and words beginning with a specific letter, named phonemic fluency. Due to the time window of the task, sustained activation is necessary for the generation of non-overlearned responses (also called processes of energization). While search and access strategies are required during fluency tasks (4), selection mechanisms seem to be key to understanding the different mechanisms involved in phonemic and semantic fluency. Phonemic fluency tasks imply a selection effort to retrieve words according to the initial letter, instead of semantic fields that are more common. Thus, associated stored words could be easily activated and should be inhibited based on task instruction. In contrast, a semantic cue would activate interconnected words based on lexico-semantic networks, giving as a result, less competition between correct words and intrusions than in a phonemic fluency task (5, 6). For this reason, deficits in phonemic fluency tasks have been more closely associated with executive dysfunction. On the other hand, deficits in semantic fluency tasks could be more related to semantic memory impairments than executive dysfunction (7). After word retrieval, self-monitoring processes play a significant role in verbal fluency tasks (4, 7).

Although verbal fluency tasks have been more studied than other fluency tasks, the cognitive processes involved in VF remain unclear (5). In this regard, the limited information of a total score (number of correct answers) has given rise to the study of other scores, such as word production during the first 15 s and errors. The higher number of words during the first 15 s, compared to the decrease in word production during the rest of the time window suggests easy access to the lexico-semantic storage and the need for a search strategy to continue the word production over the course of the task (8). Errors have also been proposed as complementary information in VF. Repetitions and intrusions (also called rule break errors) are the most frequent and have been associated with inhibition impairments (8). However, these scores do not give specific information about the lexical access strategy (9) and there is not a significant difference in error score between MS patients and healthy controls to consider errors as an optimal executive dysfunction measure (8). For a deeper understanding of the lexical access strategy, it has been proposed the study of clustering and switching (7). During task performance, participants generate different responses that can be classified into subcategories or clusters. Once a subcategory is exhausted, participants switch to a different subcategory (7). Thus, it is possible to obtain the number of clusters and switches, as well as qualitative information. These parameters could be more sensitive to detect the underlying neuropsychological deficits involved in each patient and could contribute to the understanding of cognitive function in patients with MS (10).

The cognitive profile of MS is generally characterized by impairments in processing speed, attention-executive functioning, and memory. VF tasks have some important advantages in clinical practice. They are easy to administer and shorter than other neuropsychological assessments for MS. Because VF has been associated with executive functioning and memory and they are assessed in a limited time, VF could serve as sensitive and brief cognitive function measures in MS, with special interest during early onset of the disease (11). Furthermore, these tasks are well-tolerated and are not significantly impacted by visual or motor impairments (4, 6). However, previous studies show inconsistent evidence (6). On the one hand, some authors have suggested VF as a screening test (9, 12, 13). On the other hand, while some studies suggested an equal impairment between phonemic and semantic fluency (6, 14), others have found a greater impairment in phonemic or semantic fluency (6, 15).

Our hypothesis is that VF reflects multiple cognitive components, and the assessment of different VF tasks and several parameters (number of words, clustering, switching, etc.) could be useful to disentail the cognitive demands underlying each task and score. A comprehensive assessment of VF tasks could be useful to detect patients with cognitive impairment in MS, and the impairment of specific cognitive domains, particularly executive functioning. Accordingly, our aim was 2-fold: first, to evaluate the cognitive processes related to verbal fluency in patients with MS through the identification of predictor variables of verbal fluency scores in a comprehensive neuropsychological battery; second, to develop machine-learning algorithms to predict those patients with cognitive deficits using only VF-derived scores.

MATERIALS AND METHODS

Participants

Two hundred participants with multiple sclerosis (MS) were enrolled in this study, including 146 patients with relapsing-remitting MS (RR), 19 patients with primary progressive MS (PP), and 35 patients with secondary progressive MS (SP). Main demographic and clinical characteristics are shown in **Table 1**. All participants met the McDonald 2017 criteria (16).

Neuropsychological Assessment

All participants were evaluated using the neuropsychological battery Neuronorma (17, 18), previously validated for MS in our setting. This battery included the following tests: Digit Span forward and backward, Corsi's Test forward and backward, Trail Making Test A and B (TMT), Symbol Digit Modalities

TABLE 1 | Demographic and clinical characteristics.

	MS ($n = 200$)	RR ($n = 146$)	PP ($n = 19$)	SP ($n = 35$)
Age, years	47.12 ± 9.79	44.85 ± 8.74	54.84 ± 10.44	52.28 ± 9.66
Female, %	70.1%	73.3%	42.1%	72.2%
Education, years	15.28 ± 3.76	15.58 ± 3.57	14.47 ± 3.90	14.5 ± 4.36
EDSS	3.07 ± 1.98	2.26 ± 1.43	4.76 ± 1.46	5.44 ± 1.65
Beck depression invent	13.5 ± 9.34	13.06 ± 9.78	11.89 ± 8.23	14.75 ± 7.98
Fatigue severity scale	44.70 ± 14.95	43.34 ± 15.43	44.57 ± 14.04	50.25 ± 12.26
Year of first relapse	2,001 ± 7.12	2,002 ± 6.88	2,003 ± 6.26	1,996 ± 6.82

Descriptive data are shown as mean ± standard deviation. MS, multiple sclerosis patients; RR, relapsing-remitting MS patients; PP, primary-progressive MS patients; SP, secondary-progressive MS patients.

Test (SDMT), Boston Naming Test (BNT), Rey-Osterrieth Complex Figure (ROCF) (copy, free recall after 3 and 30 min delay, and a recognition task), Judgement Line Orientation test (JLO), Stroop Color-Word Interference Test (A: word, B: color, C: interference), Free and Cued Selective Reminding Test (FCSRT) (trial 1 free recall, total free recall, total recall (free recall + cued recall) delayed recall, delayed total recall), Tower of London-Drexel test (ToL) (total moves score, total correct score, total initiation time score, total execution time score, total problem-solving time score), a semantic fluency task (SF) (animals), and a phonemic fluency tasks (PF) (words beginning with "p"). According to this battery, patients were classified as cognitively impaired or cognitively preserved using the previously validated criteria (17). In brief, these criteria define cognitive impairment when at least two cognitive domains are −1.67 standard deviations below the mean, according to age-, sex-, and education-adjusted scores. Similarly, cognitive domains were considered impaired according to the same criteria (17) (see **Supplementary Material 1**).

Furthermore, Paced Auditory Serial Addition Test (PASAT) and two extra phonemic fluency tasks (words beginning with "m" and "r") were also performed. In the VF tasks, participants were asked to produce as many words as possible in 1 min, according to the specified cues. One point was assigned for each correct word based on the guidelines by Ledoux et al. (19). In addition, Beck's Depression Inventory (20), and Fatigue Severity Scale (21) were administered.

Procedure

Patients were evaluated on a single session lasting ∼120 min. First, digit span, Corsi's test, and VF tasks were performed and took ∼10 min. Next, FCSRT was administrated and, to avoid the interference of other verbal stimuli during the delay, tests without a high verbal load were performed, such as SDMT, TMT, ROCF copy, Stroop, ROCF recall after 3 min, and ToL. FCSRT took ∼15 min with a delay of 30 min. The SDMT and Stroop were considered timed tests with time of performance of 90 and 45 s per each Stroop part, respectively. TMT, ROCF copy, and recall after 3 min took ∼7 min (for mean time details, see **Table 2**), while Tower of London test took ∼20 min. After the delayed recall of FCSRT and during ROCF 30 min delay, tests with verbal responses were administrated, such as PASAT and BNT with a mean duration of 8 and 15 min, respectively. Then, ROCF recall after 30 min, ROCF recognition task, and JLO were administered. Both ROCF tasks took ∼7 min, and JLO had a mean time of administration of 15 min. Finally, patients completed the Beck's Depression Inventory and the Fatigue Severity Scale.

All scores obtained from fluency tasks were calculated by two of the authors working independently, and final scores were reached by consensus, according to the scoring criteria developed by Ledoux et al. (19). VF-derived scores included: (a) number of correct answers without repetitions or intrusions; (b) repetitions; (c) intrusions; (d) number of clusters; (e) number of switches; (f) mean clusters (total words in clusters/ number of clusters); (g) percentage of correct words in clusters; (h) correct words in clusters. In PF, the results related to words beginning with "p" were considered singly, as well as in the sum of the results from PF considering the three initial-letters ("p," "m," and "r"), as previous studies (22).

Statistical Analysis

Statistical analysis was performed using SPSS Statistics 22.0. Descriptive data are shown as mean ± standard deviation. Pearson's correlation coefficient (r) was used for the analysis of the correlation between quantitative variables. The Pearson r coefficient was classified as very low (0–0.29), low (0.3–0.49), moderate (0.5–0.69), high (0.7–0.89), and very high (0.9–1). R software (ggplot) was used to create a heatmap of the correlation matrix. One-way ANOVA and Tukey *post hoc* test were calculated for intergroup differences, considering statistically significant a $p < 0.05$. Automatic linear modeling (LINEAR) procedure was used to identify the neuropsychological tests predictors of VF scores (23). A different model was estimated for each VF score, introducing all Neuronorma tests, PASAT, and phonemic fluency scores as predictor variables. Only variables with $p < 0.05$ were considered predictors.

Machine Learning Analysis

Two supervised classification algorithms, Support Vector Machine (SVM) with linear kernel and Random Forest (RF) were implemented with Scikit-learn v.0.22.1 in Python v.3.6.9. Six different binary classification tasks were performed depending on the class to predict: the presence of cognitive impairment or cognitive dysfunction in five different cognitive domains (attention and executive functioning, information processing speed, memory, visuospatial function, and language), according

TABLE 2 | Main neuropsychological results by the three sub groups.

Test	RR		PP		SP	
	Mean	SD	Mean	SD	Mean	SD
Span verbal (F)	6.17	1.27	5.69	1.19	6.11	1.19
Span verbal (B)	4.33	1.03	3.94	.92	4.16	1.25
Corsi's test (F)	5.72	1.00	5.25	1.07	5.53	0.964
Corsi's test (B)	4.99	1.12	4.53	1.02	4.74	0.933
TMT-A	44.58	22.54	70.82	52.83	69.61	48.12
TMT-B	94.15	50.79	131.16	83.09	206.72	236.64
SDMT	40.52	13.33	28.64	12.61	27.00	14.61
BNT	52.24	4.96	49.56	7.40	50.95	5.88
JLO	21.45	4.38	20.25	5.55	18.59	6.35
FCSRT-1FR	9.79	2.23	8.17	2.72	8.21	2.32
FCSRT-TFR	32.06	6.57	26.03	9.13	28.05	8.45
FCSRT-TR	44.30	4.93	39.94	8.90	40.32	6.96
FCSRT-FDR	11.17	3.10	8.28	3.73	8.79	4.14
FCSRT-TDR	14.76	2.01	12.56	3.91	13.21	3.29
ROCF-copy	33.32	5.31	31.51	5.17	29.63	7.95
ROCF-time	147.27	67.42	183.31	93.88	218.84	185.98
ROCF-3 min	15.98	6.83	13.28	6.69	13.89	6.88
ROCF-30 min	15.80	6.60	12.67	6.80	12.92	7.50
ROCF-recog.	19.34	2.10	19.45	1.95	19.37	1.97
Semantic fluen.	21.67	5.69	17.75	6.14	20.73	8.60
P fluency	15.33	5.36	14.47	6.24	15.05	6.83
M fluency	13.11	4.92	11.97	5.41	13.68	6.27
R fluency	13.00	4.79	11.77	4.47	14.38	6.74
Stroop-A	100.66	19.36	82.34	23.99	90.18	27.74
Stroop-B	66.24	13.22	54.03	14.85	61.71	20.78
Stroop-C	39.36	11.76	30.23	11.28	33.00	15.92
ToL-CM	4.56	2.16	3.97	2.62	3.33	2.84
ToL-TM	27.01	20.13	26.03	21.85	32.21	19.78
ToL-IT	79.74	49.28	90.59	69.11	69.64	34.40
ToL-ET	260.28	122.16	348.86	363.31	296.29	121.80
ToL-RT	337.11	127.24	365.86	170.64	365.93	141.03
PASAT-C	44.06	10.55	37.78	11.75	46.38	7.63

F, forward; B, backward; FCSRT-1FR, Free and Cued Selective Reminding Test (FCSRT) first recall; FCSRT-TFR, FCSRT total free recall; FCSRT-TR, FCSRT total recall; FCSRT-FDR, FCSRT free delayed recall; FCSRT-TDR, FCSRT total delayed recall; ROCF-recog, ROCF recognition task; Semantic fluen, semantic fluency (animals); ToL-CM, Tower of London (ToL) number of correct moves; ToL-TM, ToL number of total moves; ToL-IT, ToL initiation time; ToL-ET, ToL execution time; ToL-RT, ToL resolution time; PASAT-C, number of correct answers.

to the criteria explained above. Before performing classification, high and very high correlated features -those with a Pearson's coefficient >0.7- were excluded. For each classification task, the dataset was randomly split into training ($n = 140$, 70%) and test ($n = 60$, 30%) sets. The split was made taking into account the distribution of each class. Best hyperparameters of each model were determined carrying out a 5-Fold Cross-Validation Grid Search on the training set. Each best model was then evaluated on its corresponding test set. Models' performance was evaluated in terms of precision, recall, and F1-score values.

Ethical Approval

The study was conducted with the approval of our hospital's Ethics Committee, and all participants gave written informed consent.

RESULTS

VF Across Groups and Correlation With Non-cognitive Characteristics

Considering the classification of MS patients, there was only a significant difference between groups in semantic fluency total scores ($F_2 = 5.39$; $p = 0.005$). Tukey *post hoc* test showed differences between RR and SP groups with lower scores in SP ($p = 0.004$).

Semantic fluency total score correlated with EDSS score ($r = -0.284$; $p < 0.001$). Phonemic fluency ("p" and "pmr" total scores) also correlated with EDSS ($r = -0.208$; $p = 0.003$ and $r = -0.191$; $p = 0.008$, respectively). There was a significant correlation between semantic fluency total score and depression ($r = -0.195$; $p = 0.006$). Phonemic fluency with "p" total score ($r = -0.176$; $p = 0.012$) and phonemic fluency with

"pmr" total score ($r = -0.210$; $p = 0.003$) also correlated with depression.

Correlation Between VF and Other Neuropsychological Tests

Main neuropsychological results by the three sub groups are shown in **Table 2**. Correlations between VF and neuropsychological tests are shown in **Figure 1**. In summary, semantic fluency showed moderate correlations with BNT, FCSRT, phonemic fluency scores, SDMT, Stroop A, and Stroop B. Phonemic fluency with "p" correlated moderately with BNT, SDMT, and semantic fluency. Similar correlations were found in phonemic fluency with "pmr," including a moderate correlation with Stroop A.

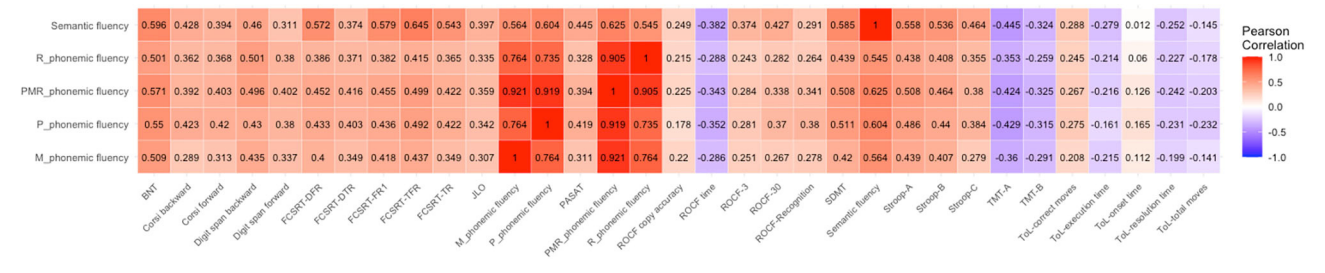

FIGURE 1 | Heatmap of Pearson correlations between fluency tasks and the other neuropsychological tests.

TABLE 3 | Automatic linear modeling assessing the neuropsychological predictors of semantic fluency.

Test	R^2	Variables (transformed)	Beta coefficient	SE	*t*	95% CI	*P*	Importance
Correct answers	0.546	Intercept	−16.895	3.257	−5.18	−23.3, −10.4	<0.001	–
		FCSRT-TFR	0.273	0.058	5.45	0.17, 0.37	<0.001	0.412
		BNT	0.348	0.072	4.84	0.20, 0.49	<0.001	0.324
		Stroop A	0.057	0.017	3.43	0.02, 0.09	0.001	0.164
		PASAT	0.077	0.037	2.08	0.004, 0.14	0.038	0.060
Repetitions	0.117	ToL-TM	0.017	0.004	3.86	0.008, 0.026	<0.001	0.467
		Stroop B	0.020	0.006	3.59	0.009, 0.032	<0.001	0.398
Intrusions	0.05	JLO	0.008	0.003	2.52	0.002, 0.015	0.012	0.443
		BNT	−0.006	0.003	−2.19	−0.012, −0.001	0.029	0.334
Clusters	0.223	Stroop A	0.015	0.006	2.31	0.002, 0.02	0.022	0.286
		Corsi (F)	0.250	0.118	2.11	0.01, 0.48	0.036	0.239
		FCSRT-TFR	0.032	0.016	2.00	0.001, 0.06	0.046	0.216
Switches	0.199	Stroop A	0.030	0.010	2.94	0.01, 0.05	0.004	0.321
		ToL-TM	0.031	0.012	2.55	0.007,0.055	0.011	0.235
		PASAT	0.061	0.024	2.52	0.01, 0.10	0.013	0.229
Mean clusters	0.138	BNT	0.077	0.027	2.88	0.02, 0.13	0.004	0.214
		FCSRT-DFR	0.168	0.060	2.82	0.05, 0.28	0.005	0.204
		TMT-A	0.017	0.006	2.76	0.005, 0.02	0.006	0.195
		Rey-copy	0.093	0.037	2.50	0.02, 0.16	0.013	0.160
		Corsi (F)	−0.319	0.128	−2.49	−0.57, −0.06	0.014	0.159
%words in clusters	0.078	Intercept	46.96	9.827	4.77	27.58, 66.34	<0.001	–
		FCSRT-TR	0.650	0.190	3.41	0.27, 1.02	0.001	0.436
		ToL-PST	0.017	0.007	2.38	0.003, 0.03	0.018	0.213
		ToL-TC	0.879	0.370	2.37	0.15, 1.60	0.018	0.212
Words in clusters	0.463	Intercept	−17.034	3.636	−4.68	−24.20, −9.86	<0.001	–
		FCSRT-TFR	0.268	0.057	4.73	0.15, 0.37	<0.001	0.349
		Stroop B	0.153	0.036	4.24	0.08, 0.22	<0.001	0.280
		BNT	0.329	0.082	4.00	0.16, 0.49	<0.001	0.249

First column shows criterion variables and the third column shows predictor variables. SE, Standard Error; FCSRT-TFR, Free and Cued Selective Reminding Test (FCSRT)–total free recall; BNT, Boston Naming Test; ToL-TM, Tower of London (ToL)–total moves; JLO, judgment line orientation; Corsi (F), Corsi's test forward; FCSRT-DFR, FCSRT–delayed free recall; TMT-A, Trail Making Test part A; Rey-Copy, Rey-Osterrieth Complex Figure–accuracy on copy; FCSRT-TC, FCSRT–total recall; ToL-PST, ToL–problem-solving time; ToL-TC, ToL–total correct moves.

TABLE 4 | Automatic linear modeling assessing the neuropsychological predictors of phonemic fluency (p).

Test	R^2	Variables (transformed)	Beta coefficient	SE	*t*	95% CI	*P*	Importance
Correct answers	0.445	Intercept	−23.43	3.879	−6.04	−31.08, −15.78	<0.001	–
		BNT	0.276	0.074	3.72	0.13, 0.42	<0.001	0.233
		ToL-IT	0.026	0.007	3.52	0.01, 0.04	0.001	0.209
		Stroop A	0.072	0.021	3.44	0.03, 0.11	0.001	0.201
		Corsi (F)	1.037	0.388	2.67	0.27, 1.80	0.008	0.121
		FCSRT-TFR	0.106	0.051	2.09	0.006, 0.20	0.038	0.074
		Rey-Recog	0.334	0.168	1.99	0.004, 0.665	0.048	0.067
Repetitions	0.041	Intercept	0.976	0.324	3.01	0.33, 1.61	0.003	–
		Stroop B	0.009	0.004	2.20	0.001, 0.01	0.029	0.431
		Corsi (B)	−0.125	0.063	−1.99	−0.24, −0.002	0.047	0.353
Intrusions	0.089	Rey-time	0.001	0.000	2.73	0.00, 0.001	0.007	0.285
		Rey-copy	0.012	0.005	2.25	0.001, 0.02	0.025	0.194
		ToL-ET	−0.000	0.000	−2.21	−0.001, 0.00	0.028	0.187
		Span (F)	−0.038	0.018	−2.12	−0.072, −0.003	0.034	0.172
		JLO	−0.008	0.004	−2.06	-0.017, 0.00	0.040	0.162
Clusters	0.204	ToL-ET	−0.003	0.001	−2.95	−0.005, −0.001	0.004	0.365
		FCSRT-TFR	0.038	0.015	2.49	0.008, 0.069	0.013	0.261
Switches	0.307	Intercept	−17.519	3.54	−4.93	−24.51, −10.52	<0.001	–
		BNT	0.210	0.056	3.75	0.10, 0.32	<0.001	0.216
		Stroop A	0.051	0.015	3.43	0.02, 0.08	0.001	0.180
		Corsi (F)	0.833	0.288	2.89	0.26, 1.40	0.004	0.129
		Tol-IT	0.026	0.009	2.87	0.008, 0.04	0.005	0.126
		ToL-ET	0.018	0.007	2.66	0.005, 0.03	0.008	0.109
		FCSRT-1FR	0.292	0.119	2.44	0.05, 0.52	0.015	1.092
		ToL-PST	−0.016	0.007	−2.29	−0.03, −0.002	0.023	0.081
		TMT-B	0.014	0.007	2.10	0.001, 0.02	0.036	0.068
Mean clusters	0.097	Intercept	4.106	0.586	7.00	2.94, 5.26	<0.001	–
		Rey-time	0.005	0.002	3.19	0.002, 0.009	0.002	0.350
		TMT-B	−0.007	0.003	−2.39	−0.013, −0.001	0.018	0.197
		Stroop C	−0.021	0.009	−2.21	−0.039, −0.002	0.028	0.168
		TMT-A	−0.012	0.006	−2.14	−0.024, −0.001	0.037	0.152
% words in clusters	0.136	Intercept	145.94	21.73	6.71	103.08, 188.81	<0.001	–
		Stroop C	−0.59	0.165	−3.58	−0.91, −0.26	<0.001	0.356
		TMT-A	−0.243	0.093	−2.63	−0.42, −0.06	0.009	0.191
		Rey-copy	−1.397	0.547	−2.55	−2.47, −0.31	0.011	0.181
Words in clusters	0.300	ToL-IT	0.040	0.009	4.59	0.02, 0.05	<0.001	0.239
		ToL-PST	−0.014	0.003	−4.54	−0.02, −0.008	<0.001	0.234
		Stroop C	−0.148	0.042	−3.51	−0.23, −0.06	0.001	0.140
		BNT	0.232	0.071	3.25	0.09, 0.37	0.001	0.120
		TMT-A	−0.055	0.018	−3.02	−0.09, −0.01	0.003	0.103
		Span (F)	0.730	0.279	2.61	0.17, 1.28	0.010	0.077
		Stroop B	0.080	0.034	2.35	0.01, 0.14	0.020	0.062

First column shows criterion variables and the third column shows predictor variables. SE, Standard Error; BNT, Boston Naming Test; ToL-IT, Tower of London (ToL)–initiation time; Corsi (F), Corsi's test forward; FCSRT-TFR, Free and Cued Selective Reminding Test (FCSRT)–total free recall; Rey-Recognition, Rey-Osterrieth Complex Figure (ROCF)–recognition; Corsi (B), Corsi's test backward; Rey-time, ROCF–time on copy; Rey-copy, ROCF–accuracy on copy; ToL-ET, ToL–execution time; Span (F), verbal span forward; JLO, judgement line orientation; FCSRT-1FR, FCSRT–trial 1 free recall; ToL-PST, ToL–problem-solving time; TMT-B, Trail Making Test part B; TMT-A, Trail Making Test part A.

Neuropsychological Predictors of VF Tests

Automatic linear modeling assessing the neuropsychological predictors of each verbal fluency score is shown in **Tables 3–5**. The criterion variables with the highest percentage of explanation by the predictor variables were correct answers, clusters, switches, and words in clusters.

In semantic fluency, the linear modeling identified FCSRT (total free recall), BNT, Stroop A, and PASAT as predictors of correct answers and explained 54.6% or the variance. Regarding

TABLE 5 | Automatic linear modeling assessing the neuropsychological predictors of phonemic fluency (p, r, m).

Test	R^2	Variables (transformed)	Beta coefficient	SE	*t*	95% CI	*P*	Importance
Correct answers	0.479	Intercept	−46.12	9.18	−5.02	-64.23, -28.01	< 0.001	–
		BNT	0.868	0.179	4.85	0.51, 1.22	< 0.001	0.271
		Stroop A	0.215	0.052	4.1	0.11, 0.31	< 0.001	0.198
		ToL-IT	0.099	0.028	3.56	0.04, 0.15	< 0.001	0.146
		Span (B)	2.85	0.898	3.17	1.07, 4.62	0.002	0.116
		Stroop C	−0.273	0.098	−2.77	-0.46, -0.07	0.06	0.088
		FCSRT-1FR	0.856	0.369	2.31	0.12, 1.58	0.002	0.062
		ToL-PST	−0.045	0.021	−2.12	-0.08, -0.003	0.035	0.052
Repetitions	0.088	FCSRT-TR	−0.048	0.017	−2.92	−0.08, −0.01	0.004	0.361
		ToL-ET	0.006	0.002	2.65	0.002, 0.01	0.009	0.297
Intrusions	0.073	Intercept	1.73	0.382	4.54	0.98, 2.49	< 0.001	–
		ToL-ET	−0.002	0.000	−3.51	-0.002, -0.001	0.001	0.550
Clusters	0.293	ToL-ET	−0.007	0.002	−3.51	−0.01, −0.003	0.001	0.240
		Stroop C	−0.082	0.027	−2.98	−0.13, −0.02	0.003	0.173
		Span (B)	0.703	0.253	2.77	0.20, 1.20	0.006	0.149
		Stroop A	0.039	0.014	2.70	0.01, 0.06	0.007	0.142
		BNT	0.124	0.048	2.58	0.02, 0.21	0.010	0.130
		Corsi (F)	0.570	0.273	2.08	0.03, 1.10	0.038	0.085
		ToL-IT	0.011	0.005	2.04	0.00, 0.02	0.042	0.081
Switches	0.328	Intercept	−31.07	6.47	−4.79	−43.84, −18.29	< 0.001	–
		BNT	0.58	0.117	4.95	0.34, 0.81	< 0.001	0.464
		Stroop A	0.12	0.029	4.44	0.07, 0.18	< 0.001	0.374
		Corsi (F)	1.51	0.620	2.44	0.29, 2.74	0.015	0.113
Mean clusters	0.082	Intercept	8.46	2.04	4.15	4.44, 12.49	< 0.001	–
		FCSRT-TR	0.108	0.038	2.82	0.03, 0.18	0.005	0.335
		PASAT	0.061	0.027	2.29	0.009, 0.11	0.023	0.222
		TMT-B	−0.014	0.006	−2.18	−0.02, −0.001	0.030	0.201
%words in clusters	0.182	Intercept	193.26	24.51	7.88	144.90, 241.62	< 0.001	–
		Stroop C	−1.81	0.350	−5.19	−2.51, −1.12	< 0.001	0.513
		Span (B)	11.68	4.03	2.89	3.73, 19.64	0.004	0.160
		ToL-ET	−0.109	0.039	−2.81	−0.18, −0.03	0.005	0.151
		Rey-3 min	1.51	0.595	2.54	0.34, 2.68	0.012	0.123
Words in clusters	0.332	Stroop C	−0.430	0.107	−4.03	−0.64, −0.22	< 0.001	0.273
		ToL-ET	−0.026	0.008	−3.28	−0.04, −0.01	0.001	0.181
		ToL-IT	0.054	0.020	2.72	0.01, 0.09	0.007	0.124
		BNT	0.503	0.198	2.54	0.11, 0.89	0.012	0.108
		Stroop B	0.216	0.089	2.43	0.04, 0.39	0.016	0.099
		Span (B)	2.402	1.08	2.21	0.25, 4.54	0.028	0.082

First column shows criterion variables and the third column shows predictor variables. SE, Standard Error; BNT, Boston Naming Test; ToL-IT, Tower of London (ToL)–initiation time; Span (B), verbal span backward; FCSRT-1FR, Free and Cued Selective Reminding Test (FCSRT)–trial 1 free recall; ToL-PST, ToL–problem-solving time; FCSRT-TR, FCSRT–total recall; ToL-ET, ToL–execution time; Corsi (F), Corsi's test forward; TMT-B, Trail Making Test part B; Rey-3 min, Rey-Osterrieth Complex Figure (ROCF) free recall after 3 min.

cluster score, the model included Stroop A, Corsi's test, and FCSRT (total free recall) and explained 22.3% of the variance. For switches score, the model identified Stroop A, ToL (total moves), and PASAT as predictors, explaining 19.9% of the variance. For words in clusters, FCSRT (total free recall), Stroop B, and BNT was identified as predictors and explained 46.3% of the variance.

In phonemic fluency with "p," the linear modeling identified BNT, ToL (initiation time), Stroop A, Corsi's test, FCSRT (total free recall), and FCRO (recognition) as predictors of correct answers and explained 44.5% of the variance. For cluster score, ToL (execution time) and FCSRT (total free recall) were identified as predictors, explaining 20.4% of the variance. For switches score, BNT, Stroop A, Corsi's test, ToL (initiation, execution, and problem-solving time), FCSRT (first trial), and TMT-B were identified by the model as predictors and explained 30.7% of the variance. For words in clusters, the model included ToL (initiation, problem-solving time), Stroop C, BNT, TMT-A, verbal span, and Stroop B, explaining 30% of the variance.

In phonemic fluency with "pmr," BNT, Stroop A and C, ToL (initiation time), verbal span, FCSRT (first trial), and ToL (problem-solving time) were included and explained 47.9% of the variance. For clusters score, the model identified ToL (initiation and execution time), Stroop A and C, verbal span, BNT, and Corsi's test and explained 29.3% of the variance. For switches,

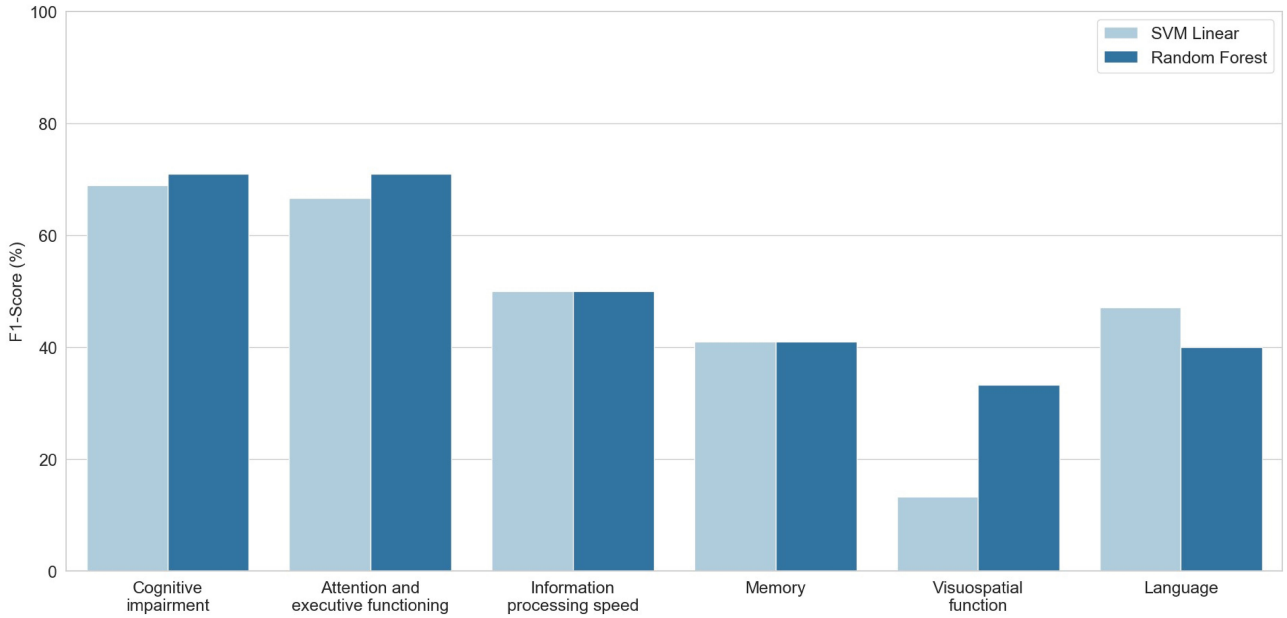

FIGURE 2 | F1-Scores (y-axis) obtained for each classification task (x-axis) using Support Vector Machine with linear kernel (SVM Linear, light blue) and Random Forest (blue) algorithms.

BNT, Stroop A, and Corsi's test were included and explained 32.8% of the variance. Finally, the model identified Stroop B and C, ToL (initiation and execution time), BNT, and verbal span as predictors, explaining 32.2% of the variance.

Machine Learning Classification

Two different classifiers (Support Vector Machine and Random Forest) were used to predict the presence of cognitive impairment, as well as the presence of cognitive dysfunction in each evaluated cognitive domain. Tuned hyperparameters and specifications of each model can be found in **Supplementary Material 2**. **Figure 2** shows the F1-score obtained for each classifier, and full information about precision, recall, and F1-score values are depicted in **Supplementary Material 3**. Both aforementioned classifiers performed better for cognitive impairment and attention and executive dysfunction, with F1-scores between 67 and 71%. Conversely, classification performance scores for the other cognitive domains were lower. Features importances in Random Forest models are shown in **Figure 3**.

DISCUSSION

The cognitive processes involved in verbal fluency in MS remains controversial, due to the specific characteristics of cognitive impairment and brain damage associated with MS. In this study, we applied automatic linear modeling to investigate the neuropsychological tests that better explained the verbal fluency tests performance. Interestingly, we found different predictors according to the different fluencies (phonemic or semantic) and the different scores used (total words, clustering, and switching). These results support the view that fluency tasks provide useful information about a wide range of cognitive functions. Specifically, semantic fluency (total score) was predicted by the FCSRT (total free recall), Boston Naming Test, Stroop A, and PASAT, which confirm the influence of memory and language tasks, but also attention and time-dependent tests. Similarly, clustering in semantic fluency was predicted by the FCSRT, Stroop A, and Corsi test. Conversely, switching in semantic fluency was mainly explained by three attention-executive and time-dependent tests: Stroop A, ToL, and PASAT.

Regarding phonemic fluency, several tests measuring attention-executive functioning, language, and memory were the main predictors. Clustering was predicted by ToL and FCSRT, while switching by BNT, Stroop A, Corsi, ToL, FCSRT, and TMT-B. Thus, our results confirm the influence of three main cognitive domains in fluency tasks, including attention-executive functioning, memory, and language. Although the tests mainly associated with these cognitive domains are predictors of the different fluencies and scores, the importance of memory was greater in semantic fluency and clustering, and executive functioning in phonemic fluency and switching. In addition, it is worth mentioning that several of the best predictors were time-dependent tasks, which also emphasize a potential role of processing speed. Although the SDMT was not included in any statistical model, it showed moderate correlations with all the fluency scores, as in previous studies (6, 9). Overall, these findings emphasize the interest to extract several parameters in fluency tasks to capture as much information as possible.

Another interesting result is the role of the Boston Naming Test, which predicted several fluency scores, such as correct answers in semantic and phonemic fluency. This test shares some

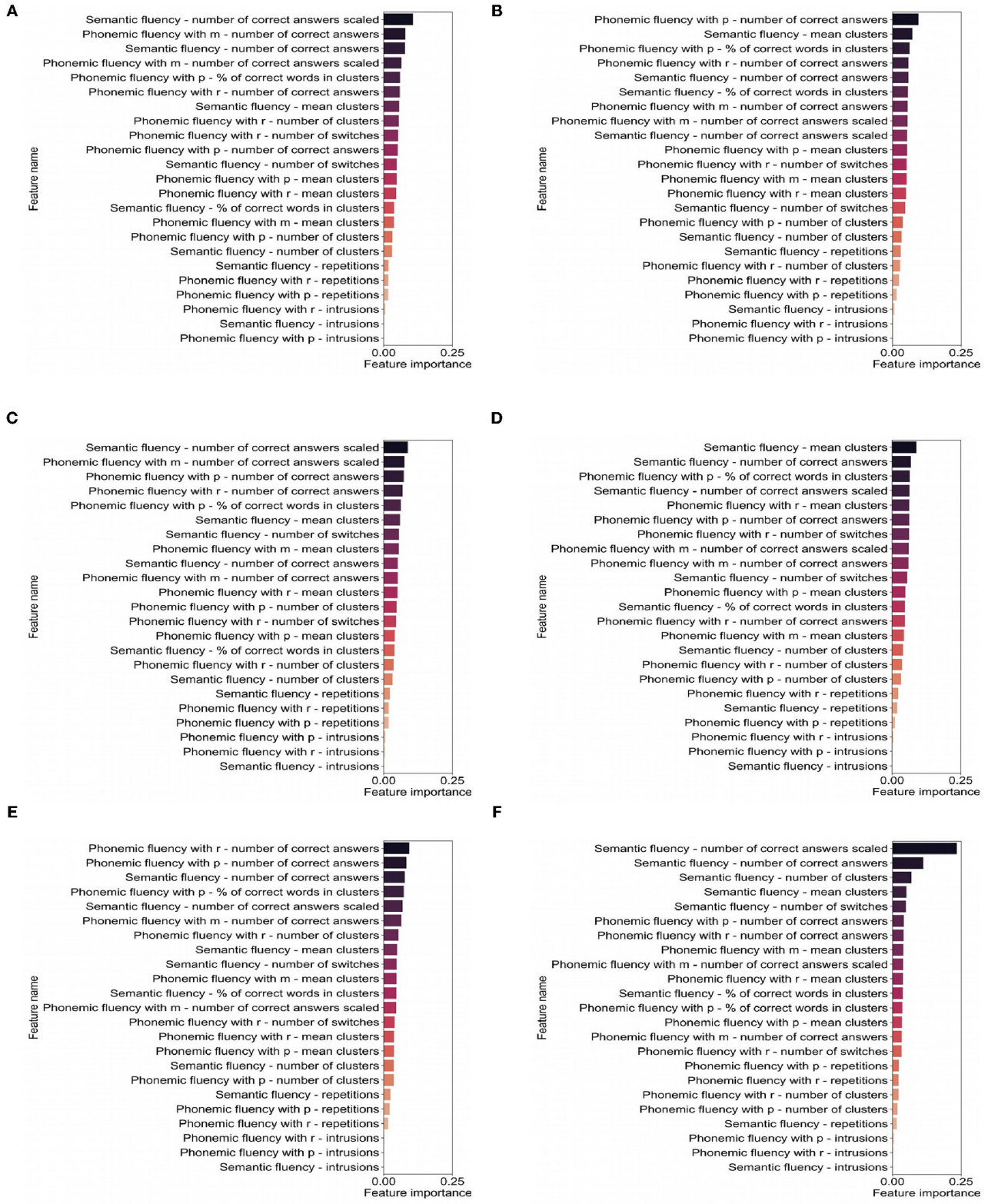

FIGURE 3 | Features importances obtained with each Random Forest model for each classification task: **(A)** cognitive impairment, **(B)** attention and executive functioning, **(C)** information processing speed, **(D)** memory, **(E)** visuospatial function, and **(F)** language.

cognitive processes with fluency tasks, such as search, selection, and word retrieval, but with a lower degree of time restriction. Although language was usually considered to be largely preserved in MS, recent studies using novel tests evaluating the speed to lexical access have shown frequent impairment even in early stages (24).

We have developed several machine-learning algorithms trying to predict those patients with cognitive impairment, and those with dysfunction of specific cognitive domains. Interestingly, VF scores achieved acceptable values for the prediction of general cognitive impairment and executive dysfunction, which confirms the major role of executive functioning in VF in MS. Scores derived from phonemic fluency (e.g., correct words beginning with "p," clusters, and switches) were more useful in the prediction of executive dysfunction. For general cognitive impairment prediction, a combination of scores from semantic and phonemic fluencies were amongst the most predictive, which suggests the interest of combining semantic and phonemic VF in short batteries (14). Unfortunately, the algorithms showed low levels of accuracy in the other cognitive domains, which supports the need for a full and comprehensive neuropsychological assessment to evaluate specific cognitive deficits in MS.

These findings may also be interpreted in terms of the neural basis of cognitive dysfunction in MS. Semantic fluency and phonemic fluency have been associated with subcortical volumes in voxel-based morphometry analysis (2). Specifically, phonemic fluency was mainly correlated with caudate, while semantic fluency with both thalamus and caudate in both hemispheres. Impairment of these structures is considered key in the pathophysiology of cognitive impairment in MS, especially in attention and executive functioning. Conversely, in other functions, such as memory or language, other regions are necessary to predict cognitive performance (i.e., hippocampus and temporal lobe in memory) (25). Neural basis of cognitive assessment in MS shows several particularities, in contrast with other disorders (tumors, stroke, or neurodegenerative dementias). In this regard, in other disorders VF has been mainly correlated with several cortical regions in the left hemisphere (26). These specificities warrant the study of the cognitive processes and neuroimaging correlates of the neuropsychological tests used in the setting of MS to accomplish an adequate interpretation of neuropsychological assessment.

Our study has some limitations. First, algorithms were developed on the basis of some criteria, which also included the impairment of VF. This could imply a certain degree of circularity in the machine learning analysis. However, these criteria were previously validated in an independent study, and impairment of VF according to these criteria was present in a relatively low percentage of cases classified as cognitively impaired (36.2% for semantic VF, and 29.8% for phonemic VF). Second, VF are tasks language-dependent, and our results should be confirmed in other cultures. In this regard, there are differences in the frequency of words between languages, and cross-cultural adaptations are required to minimize it, especially for phonemic fluency (27, 28). For instance, words beginning with "f," "a," and "s" are common phonemic fluency tasks for English speakers, but for Spanish speakers the initial letters "p," "m," and "r" have been proposed as an alternative and are generally preferred, based on the frequency of words (27–29). Third, we did not include neuroimaging analysis in this study. Correlation between the different scores and neuroimaging techniques (voxel-based morphometry, cortical thickness, diffusion tensor imaging, etc.) may be of interest in future studies. Fourth, we did not perform a correction considering motor dexterity. Due to the possibility of motor disorders in MS patients that could compromise the test interpretation, particularly in timed neuropsychological tests, this type of correction may be useful to improve the reliability of the neuropsychological examination (30). Finally, due to the aims of the study, a comprehensive battery was administrated with the possible presence of fatigue effect.

In conclusion, our study highlights the interest of further research into the assessment of VF in patients with MS. VF was influenced by many other cognitive processes, mainly including attention-executive functioning, episodic memory, and language. Semantic fluency and clustering were more explained by memory function, while phonemic fluency and switching were more related to executive functioning. The multiple cognitive components underlying VF tasks could serve for screening purposes. In this regard, we have developed several machine learning algorithms that could be useful to detect patients with cognitive impairment using only VF, although these models performed adequately only for general cognitive impairment and executive dysfunction. Overall, our study supports the implementation of a comprehensive and qualitative assessment of verbal fluency in MS, which may provide interesting insights into cognitive function in patients with MS.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comite de Etica e Investigacion Clinical del Hospital Clinico San Carlos. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AD-A, JM-G, and JAM-G: conceptualization and design of the study. AD-A, CD-A, AC-M, LV, PM-E, JM-G, and JAM-G: data curation. AD-A, LH-L, and JAM-G: formal analysis. JM-G: funding acquisitions. AD-A, CD-A, LH-L, AC-M, PM-E, VP, JM-G, and JAM-G: investigation. JM-G and JAM-G: supervision. AD-A and JAM-G: writing original draft. LH-L, CD-A, AC-M, LV, PM-E, VP, and JM-G: writing review and editing. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.629183/full#supplementary-material

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Cognitive Impairment Impacts Exercise Effects on Cognition in Multiple Sclerosis

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Purpose: Exercise training reveals high potential to beneficially impact cognitive performance in persons with multiple sclerosis (pwMS). Research indicates that high-intensity interval training (HIIT) has potentially higher effects on physical fitness and cognition compared to moderate continuous exercise. This study (i) compares the effects of a 3-week HIIT and moderate continuous exercise training on cognitive performance and cardiorespiratory fitness of pwMS in an overall analysis and (ii) investigates potential effects based on baseline cognitive status in a subgroup analysis.

Methods: Seventy-five pwMS were randomly assigned to an intervention (HIIT: 5 × 1.5-min intervals at 95–100% HR_{max}, 3 ×/week) or active control group (CG: 24 min continuous exercise at 65% HR_{max}, 3 ×/week). Cognitive performance was assessed pre- and post-intervention with the Brief International Cognitive Assessment for MS (BICAMS). (I) To examine potential within (time) and interaction (time × group) effects in the overall analysis, separate analyses of covariance (ANCOVA) were conducted. (II) For the subgroup analysis, participants were divided into two groups [intact cognition or impaired cognition (>1.5 standard deviation (SD) compared to healthy, age-matched norm data in at least one of the three tests of the BICAMS]. Potential impacts of cognitive status and intervention were investigated with multivariate analyses of variance (MANOVA).

Results: Overall analysis revealed significant time effects for processing speed, verbal learning, rel. VO2peak, and rel. power output. A time*group interaction effect was observed for rel. power output. Subgroup analysis indicated a significant main effect for cognition (impaired cognition vs. intact cognition). Subsequent *post-hoc* analysis showed significant larger effects on verbal learning in pwMS with impaired cognition.

Conclusion: Current results need to be confirmed in a powered randomized controlled trial with cognitive performance as primary endpoint and eligibility based on cognitive performance that is assessed prior to study inclusion.

Keywords: cognitive performance, exercise, processing speed, verbal learning, visuospatial memory, high-intensity exercise

INTRODUCTION

Cognitive impairment represents a common and debilitating symptom in multiple sclerosis (MS). Forty-three percent to 70% of persons with MS (pwMS) experience cognitive impairment, predominantly characterized by slowed processing speed and impaired memory function (1). Since reduced physical ability is often described as a hallmark of MS symptomology, cognitive impairment tends to lose focus in everyday care. Nevertheless, impaired cognition has a profound impact on peoples' working and driving ability and on their overall quality of life (2).

Existing pharmacological treatments target a reduction of disease activity by modifying the immune system and its effects on the central nervous system (CNS). A few of these disease-modifying drugs reveal cognition-enhancing effects (3). However, they are not generally effective in counteracting cognitive impairment (4). Moreover, symptomatic treatments that are used for dementia are not, or only marginally effective for cognitive impairment in pwMS (5). Against this backdrop, investigations on novel non-pharmacological treatment options gain focus in current research.

Exercise training especially became of particular interest as a non-pharmacological supportive treatment option in the last decade. Previous research has already shown associations between exercise training and improved cognitive performance in healthy and cognitively impaired older adults (4, 6, 7). Additionally, data also suggest exercise-induced neuroprotective effects in several neurological diseases, such as Alzheimer's disease (8).

In contrast, little is known about the effects of exercise training on degenerative CNS processes in MS and its impact on cognitive impairment. Currently, research on this topic is growing and several approaches investigating potential beneficial effects of exercise for pwMS have been initiated. Research indicates positive associations between an increased cardiovascular fitness (VO2peak) and larger volumes of deep gray matter structures, involving the hippocampus (9). The hippocampus is indeed mainly responsible for memory and learning, functions that are commonly affected in MS. Another study revealed increased cortical thickness following an exercise training intervention, indicating neuroprotective and potential neuroregenerative effects of exercise (10). In fact, high-intensity interval training (HIIT) has been described to potentially induce greater enhancements in cardiorespiratory fitness than moderate continuous exercise in pwMS (11). Moreover, Zimmer et al. (12) showed in a previous randomized controlled trial (RCT) that HIIT significantly improved verbal learning compared to a moderate continuous control group (CG).

On a functional level, a growing body of literature has investigated the effects of exercise training on cognitive performance in pwMS. However, existing results remain contradictory, since some studies report beneficial impacts on specific cognitive domains such as verbal learning (12) while others demonstrate non-significant results (13). Overall, evidence of exercise studies on cognitive performance in pwMS is still sparse. A recent meta-analysis evaluating the effects of exercise training on global cognitive performance and MS-specific cognitive domains (processing speed, learning/memory, executive functions, and attention) (14) did not identify any significant effects. This work supports the conclusions of a former meta-analysis and review (15, 16) with regard to several, still emerging, methodological limitations of existing studies. In addition to many other limitations, most of the existing studies investigating exercise-induced effects on cognitive performance do not focus on screening participants' cognitive performance prior to inclusion.

The objective of this study is to analyze the effects of a HIIT and moderate continuous exercise on cognitive performance in pwMS. Since cognitive performance was a secondary outcome of this RCT (17), the above mentioned limitation of participants not being included based on their cognitive impairment is given. In order to go one step further and consider this limitation, we not only investigate (i) the effect of HIIT on cognitive performance of the total sample (overall analysis) but additionally (ii) conduct a subgroup analysis (total sample subdivided based on baseline cognitive status) in order to achieve more meaningful results on this secondary outcome.

METHODS

Study Design and Overview

The original study is a RCT with a parallel (1:1) group design and primarily investigated the change of proportions of circulating T-regulatory cells (Tregs) over a 3-week intervention period comparing HIIT vs. CG. The study was approved by the regional ethics committee (EKOS18/96; Project ID: 2018–01378), registered at ClinicalTrials.gov (NCT03652519; August 29, 2018) prior to recruitment start and conducted in accordance with the principles of the Declaration of Helsinki. Details on methods and all outcomes that are not relevant for the present investigation are shown elsewhere (17). This publication presents an analysis of this RCT with special interest on the secondary outcome cognitive performance.

Participant Recruitment and Eligibility

Participant recruitment, testing, and exercise intervention were conducted in the inpatient rehabilitation clinic Valens (Switzerland). Inpatients were screened for eligibility over a 12-month period (October 2018–October 2019). All inpatients received a comprehensive medical check on the day of admission. Persons >21 years old holding a definite MS diagnosis [according to the revised McDonald criteria (18)] with a relapsing–remitting or secondary progressive disease course and an Expanded Disability Status Scale (EDSS) score between 3.0 and 6.0 (inclusive) fulfilled the key inclusion criteria. Persons with concomitant diseases (internistic, orthopedic, neurological, acute melanoma, and cancer), acute relapses, or disease worsening immediately before study start, limiting the participation in the exercise intervention or affecting study outcomes, were excluded. Moreover, non-German-speaking persons and persons with diagnosed psychological disorders were excluded, since the understanding of study course and execution of instructions

could be affected. Pregnancy or breast feeding, drug or alcohol abuse, and persons employed for study execution were also criteria for study exclusion (17). Additionally, participants who experienced acute relapses or received immune-modulatory medication the day prior to cardiopulmonary exercise testing (CPET) were excluded. In case participants developed acute unwellness over the study period, exercise sessions were canceled on that day and if possible conducted on another day of the week. Participants were informed about the study and gave their written consent before inclusion.

Randomization and Masking

After baseline assessment, participants were randomized (1:1) into an exercise intervention group or CG. A concealed randomization was conducted with the "Randomization-In-Treatment-Arms" software (RITA, Evident, Germany). Cardiorespiratory fitness (assessed by CPET), disease severity (EDSS score), age, and fatigue [Fatigue Scale for Motor and Cognitive Functions (FSMC) (19)] were applied as factors for stratification. For all stratification factors, separate ranges were defined in the randomization software prior to first randomization (EDSS: 3/3.5, 4/4.5, 5/5.5, 6; cardiorespiratory fitness: $<$100 W, $\geq$100 W, age: 20–29, 30–39, 40–49, 50–59, 60–69, 70–80, fatigue: $<$43, $\geq$43). Randomization was carried out by a researcher at the German Sport University Cologne, who was not involved in the study procedures during data recruitment. CPET was conducted by the principal investigator who was blinded to the training condition.

Exercise and Control Group Treatment

The exercise interventions consisted of aerobic endurance training sessions on a bicycle ergometer. Both groups exercised three times a week for 3 weeks. Exercise intensity was heart rate controlled based on the highest heart rate (HR_{max}) achieved at baseline CPET. Each session comprised a 3-min warm-up and cool-down period at low intensity [50% maximum heart rate (HR_{max})]. Besides the exercise intervention, participants of both groups received the regular individual rehabilitation program of the Valens clinic.

Experimental Intervention Group (HIIT)

The exercise group performed five 1.5-min high-intensity intervals at 95–100% of HR_{max} with 80–100 rpm. Between the intervals, active breaks of 2 min unloaded pedaling were conducted, aiming to achieve 60% HR_{max}.

Control Group Treatment

Participants assigned to the CG exercised continuously three times a week for 24 min at 65% of HR_{max} with 60–70 rpm. This intervention represents the usual exercise regime of the Valens clinic and can be described as a standard care active control regime.

Outcome Measures

Outcome measures were assessed after the day of clinical admission, prior to intervention start (T0) and at discharge of the 3-week intervention (T1).

Aerobic Fitness

Participants performed a graded cardiopulmonary exercise (Jaeger CPX, Germany) test at T0 and T1 on a bicycle ergometer (Ergoline 800, Germany) until a participants' symptom reached maximum (e.g., muscular fatigue). Peak oxygen consumption (VO2peak), maximum workload (watts), and heart rate [beats per minute (bpm)] were assessed during the test. The protocol started with 3 min of rest (no pedaling), 3 min of unloaded pedaling (warm-up), followed by the testing, and ended with 3 min of unloaded pedaling (cool-down). Workload was continuously ramp-type increased by 10 W each minute to ensure a testing phase of 8–12 min. Baseline CPET results (HR_{max}) served as the anchor for individual exercise intensities in the HIIT group and CG.

Patient-Reported Outcome Measures

Fatigue was measured with the German version of the FSMC (19) comprising 10 items for motor and 10 items for cognitive fatigue. Cutoff scores for low and high levels of fatigue were set at 43/100 for the total score, 22/50 for the motor (FSMC mot.), and 22/50 for the cognitive (FSMC cog.) subscores.

Cognitive Performance

Cognitive performance was assessed with the Brief International Cognitive Assessment for MS (BICAMS) (1) modified for the use in German language. This test battery contains three tests assessing the main cognitive domains vulnerable to MS. Processing speed is measured by the Symbol Digit Modalities Test (SDMT), verbal learning by the Verbal Learning Memory Test (VLMT), and visuospatial learning and memory by the Brief Visuospatial Memory Test-Revised (BVMT-R). The original BICAMS version recommended the California Verbal Learning Test or any verbal memory list learning task. The VLMT was used in this study, because the VLMT norm data for the German population are based on a larger sample size and include a larger age range (20). Parallel versions for two tests, the VLMT, and the BVMT-R, were applied. The BICAMS test battery represents a validated, frequently recommended and applied test battery to evaluate cognitive performance of the most commonly affected domains in pwMS. Therefore, only this assessment was used for the current analysis.

Statistical Analysis

Sample size calculation focused on detecting between group effects on the proportion of Tregs, the primary outcome of the RCT. Details on the precise process of sample size calculation are explained elsewhere (17). The final sample size for this study results in $N = 72$ participants.

In a first step, an overall analysis was conducted with separate analysis of covariance models with repeated measures and adjusted for baseline values (ANCOVA) to assess potential between-group effects (HIIT vs. CG) over time for cognitive performance, fatigue, and cardiorespiratory fitness outcomes. Therefore, "time" was defined as the within-subject factor and "group" was defined as the between-subject factor. Dependent variables were the cognitive outcomes (SDMT, VLMT, and BVMT-R), the fatigue outcome (FSMC), and the

cardiorespiratory fitness outcomes [rel. (relative) VO2peak and rel. power output]. In this analysis, the whole sample was analyzed as one.

In a second step, MAN(C)OVA was conducted to determine potential effects of cognitive status (impaired cognition vs. intact cognition) and group (HIIT vs. CG) and their interaction (group∗cognition) on changes of cognitive performance. For this subgroup analysis, the sample was divided into two groups, "impaired cognition" and "intact cognition." Participants with baseline values >1.5 standard deviation (SD) compared to healthy, age-matched norm data (21–23) in at least one of the three tests were allocated to the "impaired cognition" group. All other participants were allocated to the "intact cognition" group. For the multivariate ANOVAs, the delta values of the SDMT, VLMT, and BVMT-R were used as the dependent variable and the factors "group" and "cognition" (impaired cognition/intact cognition) were used as fixed factors. Box's Test of Equality of Covariance Matrices and Levene's Test were checked throughout the analysis. An additional MANOVA was conducted adjusted for levels of fatigue since it might be a confounding factor.

Potential baseline differences were assessed with independent *t*-tests and Fisher's exact test and univariate one-way ANOVAs. All analyses were conducted with the intention-to-treat analysis (ITT); therefore, all randomized participants were included in the analysis. Missing values were imputed with the last observation carried forward method (LOCF), using baseline values. Outliers defined as *z* scores </>3 were replaced by the cutoff value of 3 SD (mean ± 3 × SD) from the mean score of the concerned variable. Significance was defined as $p \leq 0.05$ for univariate ANOVAs and main effects of MANOVAs. Correcting for multiple testing, the significance level for the subsequent ANOVA analysis of the MANOVAs was reduced to $p \leq 0.017$. All outcome measures of the ANCOVAs and the MANOVA are presented with *p*-values, *F* (df), and effect sizes (partial η^2). All statistical procedures were conducted with SPSS 26® (IBM®, Armonk, NY, USA).

RESULTS

A total of 75 participants were included in the study and 74 participants completed this study, leading to a completion rate of 98.67%. All participants exercised and were analyzed according to their randomized group. One participant of the CG dropped out due to non-study-related health issues following a surgery prior to baseline CPET. The overview of the study flow is shown in **Figure 1**.

No adverse events occurred. One participant declined the cognitive assessments, so the total number of participants in the subgroup analysis for cognitive performance was reduced to 73. From the 74 participants that completed the study, data of cognitive performance (all three tests) and fatigue (FSMC cog. and FSMC total) were imputed each for one participant. The reason for this missing cognitive data was that one participant declined to take part in the cognitive assessments at t1 because they felt uncomfortable. Data of the FSMC were imputed, because one question was declined by the participant. Data of both subscales of the HADS are missing for one participant and data of the anxiety subscale are missing for another participant, because items were not answered. Baseline and clinical characteristics of the participants are shown in **Table 1**. Except for sex (in the subgroup analysis), no baseline differences between groups were found, neither within the overall nor the subgroup analysis (**Table 1**). In total, 70.6% of the total sample of the impaired participants were classified as impaired in only one test of the BICAMS test battery (50% in SDMT, 50% in BVMT-R). The remaining 29.4% were classified as impaired in two or more tests (14.7% in two tests, 14.7% in three tests). Eighty percent of those who were classified as impaired in two tests showed deficits in the SDMT and BVMT-R test and 20% showed deficits in the SDMT and VLMT test. With regard to the attendance rates, participants of the HIIT group reached, on average, 79%, and those in the CG reached 70% of the planned exercise sessions. Adherence rates in the subgroups were 77% for HIIT + impaired cognition, 81% for HIIT + intact cognition, 72% for CG + impaired cognition, and 67% for CG + intact cognition. This analysis was conducted based on the intention-to-treat method, consequently including all training sessions independent of the number of missed sessions. No differences between attendance rates of the groups within the overall or subgroup analysis exist (overall analysis: 0.067; subgroup analysis: 0.268). The average training intensity of the HIIT group was 98% HR_{max} and that of the CG was 77% HR_{max}. In the subgroups: HIIT + impaired cognition, 97% HR_{max}; HIIT + intact cognition, 99% HR_{max}; CG + impaired cognition, 80% HR_{max}; CG + intact cognition, 75% HR_{max}. Ninety-two percent of the exercise sessions in the HIIT group fulfilled the targeted interval time. For the CG, on average, 94% of the planned exercise was fulfilled.

Analysis of cardiorespiratory fitness (HIIT vs. CG) showed significant effects for the main factor time (time effects) for rel. VO2peak and rel. power output. Significant interaction effects (time × group) were only observed for the rel. power output. Bonferroni-corrected *post-hoc* tests showed an improvement over time for both groups in levels of rel. VO2peak (HIIT: $p < 0.001$; 95% CI [1.697; 3.371]; CG: $p < 0.001$; 95% CI [0.741; 2.461]). Moreover, *post-hoc* tests showed that the HIIT group had significant higher rel. power outputs compared to the CG at t1. ($p = 0.011$; 95% CI [0.034; 0.250]). For the outcome fatigue, no time ($p = 0.305$) or interaction effects ($p = 0.404$) could be observed. ANCOVA results are listed in **Table 2**.

Regarding outcomes of cognitive performance, two separate analyses were conducted. (I) The overall (HIIT vs. CG) analysis revealed significant time effects for processing speed (SDMT), verbal learning (VLMT), and visuospatial memory (BVMT-R) but no significant group or group × time interaction. ANCOVA results are listed in **Table 2**.

Bonferroni-corrected *post-hoc* tests showed improvements of processing speed (HIIT: $p < 0.001$; 95% CI [2.112; 5.223]; CG: $p < 0.001$; 95% CI [2.172; 5.414]) over time in both groups; VLMT and BVMT-R showed no effects. For the variables VLMT and BVMT-R, no significant results were observed after Bonferroni-corrected *post-hoc* tests {VMLT (HIIT: $p = 0.60$; 95% CI [−1.731; 2.977]; CG: $p = 0.723$; 95% CI [−2.015; 2.891]), BVMT-R (HIIT: $p = 0.577$; 95% CI [−2.114; 1.186]; CG: $p = 0.302$; 95% CI

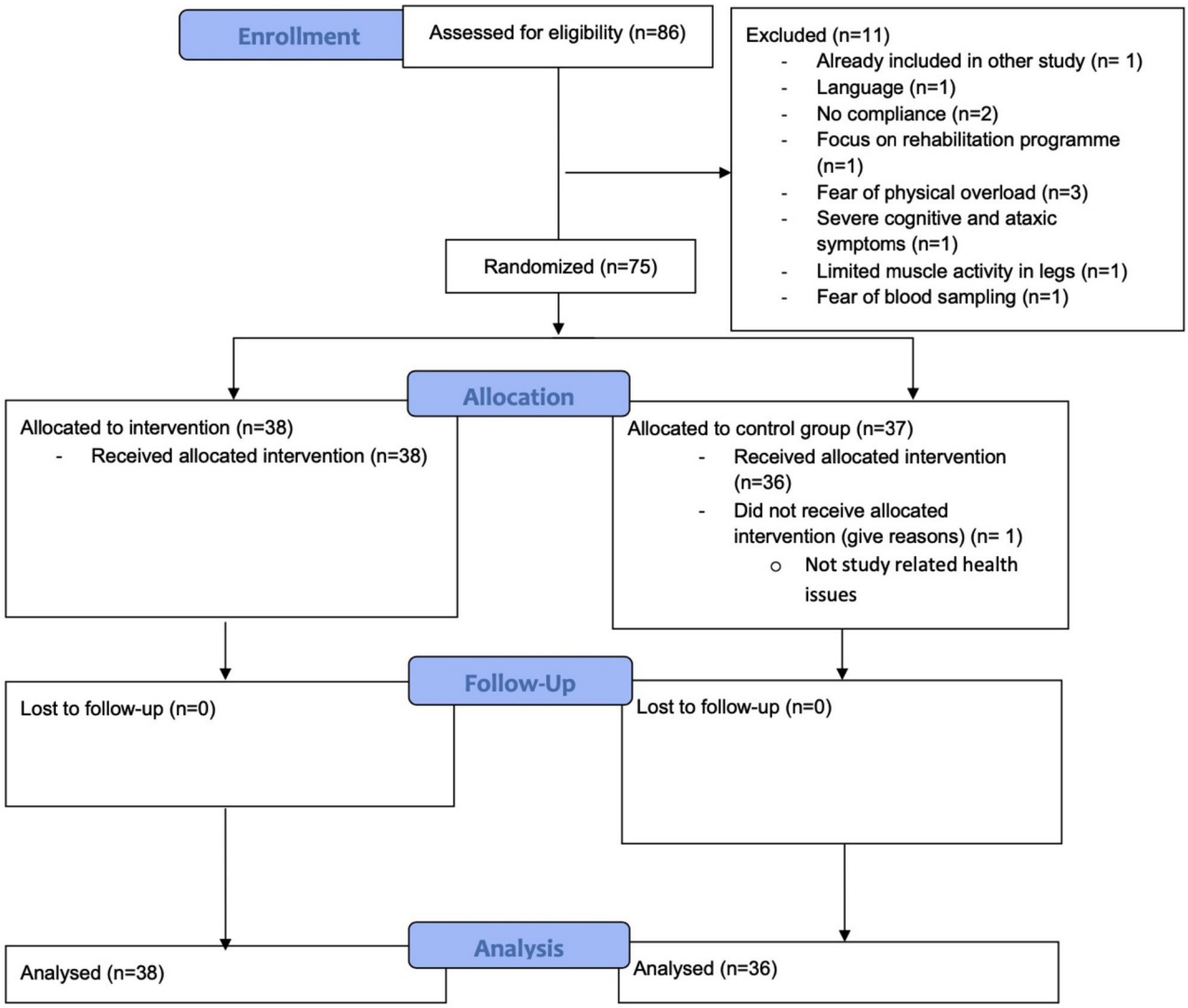

FIGURE 1 | Participant flow diagram.

[−2.616; 0.823])}. Baseline-adjusted ANCOVA results for all outcomes of the overall analysis are shown in **Figures 2**, **3**.

(II) MANOVA results of the subgroup analysis revealed a significant main effect for cognition (impaired cognition vs. intact cognition) but not for the main factor group or their interaction (cognition × group). Subsequent *post-hoc* analysis revealed significant differences between impaired cognition and intact cognition for verbal learning (impaired cognition: 95% CI [0.345; 5.455] intact cognition 95% CI [−4.121; 0.695]). Since the level of significance was corrected for multiple testing, *p*-value was reduced to 0.017. Therefore, no further significant effects were detected. However, a tendency ($p = 0.025$) could be observed for the visuospatial memory (impaired cognition: 95% CI [−0.871; 3.047]; intact cognition 95% CI [−3.855; −0.162]). Results of the subgroup analysis are listed in **Table 3** and shown in **Figure 4**. Adding the variable sex as a covariate into the model does not change any significant results. Conducting the analysis with both sex and baseline fatigue levels as a covariate, the same trend of results can be observed (**Supplementary Material 1**).

DISCUSSION

This study focused on an analysis of a secondary outcome (cognitive performance) of an original RCT by investigating (i) the effect of HIIT vs. CG on cognitive performance in an overall analysis and (ii) examining the effect of cognitive status (impaired cognition vs. intact cognition) within a subgroup analysis. Results of the overall analysis showed significant time effects for processing speed and verbal learning. Results of the subgroup analysis suggest that effects of exercise training on verbal learning are dependent on cognitive status. In detail, participants classified as cognitive impaired at baseline revealed positive changes in

TABLE 1 | Baseline characteristics of the participants.

	Overall analysis			Subgroup analysis				
				Participants with impaired cognition		Participants with intact cognition		
	HIIT ($n = 38$)	CG ($n = 36$)	p	HIIT ($n = 15$)	CG ($n = 19$)	HIIT ($n = 23$)	CG ($n = 16$)	p
Sex (f/m)	27/11	21/15	0.331	15/0	9/10	12/11	12/4	0.002*
Age (years)	51 (10.97)	49 (10.12)	0.418	50.73 (13.52)	49.26 (10.44)	51.17 (9.27)	48.31 (10.25)	0.843
MS phenotype (RRMS/SPMS)	23/15	22/14	1.000	9/6	12/7	14/9	9/7	0.987
EDSS-Score	4.5 (1.05)	4.53 (1.08)	0.911	4.67 (1.11)	4.5 (1.2)	4.39 (1.01)	4.59 (0.99)	0.876
Rel. VO2peak (ml kg^{-1} min^{-1})	19.04 (5.61)	19.25 (5.12)	0.868	16.79 (4.68)	19.38 (5.8)	20.51 (5.77)	19.04 (4.54)	0.223
Rel. power output (watts/kg)	1.34 (0.53)	1.32 (0.44)	0.866	1.22 (0.42)	1.36 (0.47)	1.43 (0.58)	1.26 (0.42)	0.559
Power output (watts)	96.59 (38.87)	95.50 (31.42)	0.894	81.33 (31.78)	97.11 (31.01)	106.55 (40.46)	90.5 (30.94)	0.163
Fatigue (FSMC)	69.45 (15.66)	66.42 (13.41)	0.373	71.28 (17.64)	70.05 (12.78)	68.26 (14.52)	63.5 (12.82)	0.448
Motor fatigue (FSMC-mot)	36.73(7.92)	35.75 (6.85)	0.571	35.85 (8.91)	36.74 (6.94)	37.3 (7.36)	35.44 (6.13)	0.863
Cognitive fatigue (FSMC-cog)	32.26 (9.68)	30.67 (7.84)	0.440	34.27 (9.88)	33.32 (6.95)	30.96 (9.54)	28.06 (8.02)	0.187
HADS (Depression subscale)	4.66 (3.31)	4.00 (3.26)	0.402	4.73 (3.37)	4.90 (4.08)	4.61 (3.35)	3.06 (1.81)	0.356
HADS (Anxiety subscale)	5.47 (3.61)	4.62 (3.62)	0.318	6.33 (3.37)	5.47 (3.94)	4.91 (3.73)	4.00 (3.10)	0.326
SDMT (points)	43.44 (9.95)	42.63 (13.76)	0.771	37.8 (8.41)	32.84 (7.23)	47.13 (9.25)	54.25 (10.04)	
VLMT (points)	52.08 (8.95)	52.37 (11.73)	0.906	48.6 (8.67)	47.37 (10.48)	54.35 (8.56)	58.31 (10.51)	
BVMT-R (points)	20.42 (7.4)	20.09 (7.95)	0.853	14.93 (5.26)	15.89 (6.91)	24 (6.37)	25.06 (6.09)	

*Data are presented as mean (standard deviation) except for sex and MS phenotype (proportions). HIIT, high-intensity group; CG, control group; MS, multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; EDSS, Expanded Disability Status Scale; rel., relative; FSMC, Fatigue Scale for Motor and Cognitive Functions; mot., motor subscale; cog., cognition subscale; HADS, Hospital Anxiety and Depression Scale. *Significant differences between groups.*

VLMT scores, compared to participants with intact cognition. However, no significant cognition × group interaction was observed. A similar trend was found for visuospatial memory; however, results did not reach statistical significance.

By conducting a subgroup analysis based on the predefined cognitive status of the participants, we considered a common limitation of the majority of exercise studies in this research context. The results strongly support the need of pre-defined inclusion criteria for cognitive performance in exercise intervention studies with pwMS. A major reason why existing studies mostly include participants without assessments of cognitive performance prior to inclusion might be that most of the existing studies do not define cognitive performance as a primary outcome. However, from a methodological point of view, the consideration of cognitive performance at baseline is necessary, as groups with heterogeneous cognitive status achieve varying results, requiring larger sample sizes (24). Baseline memory competence and information processing speed have been shown to be independent predictors of cognitive rehabilitation outcome in MS (25). These findings may partially explain the results of a recent meta-analysis that reported null effects of exercise on global and domain-specific cognitive performance in pwMS. Interestingly, out of 13 included studies only one study (26) evaluated cognitive performance prior to study inclusion. Recruitment of enriched samples of cognitively impaired PwMS are now recommended for cognitive retraining studies in MS (27).

Generally, the evidence for potential effects of exercise training on cognitive performance remains unclear, because the emerging results are inconclusive due to methodological limitations and heterogeneous exercise interventions (14, 16). In a previous published study with similar exercise interventions, significant time × group interactions for verbal learning were identified, indicating that HIIT improved VLMT scores compared to CG. These results are in line with those of Briken et al. (28) who reported significant effects of three different exercise interventions (arm ergometry, rowing, and cycling) compared to a waitlist CG on VLMT scores. The present study did not include a passive or waitlist CG treatment, as these are critical to establish from an ethical point of view in clinical settings like rehabilitation centers. Results of the current study did not confirm those of the previous investigation, which might be explained by the following reasons. The portion of pwMS with impaired cognition relative to the studies' sample size was comparable for the HIIT group. However, the CG group of the current study had a higher portion of pwMS with impaired cognition compared to the previous study indicating, based on the results, that CG also had beneficial impacts on cognitive performance, leading to no interaction effect in the current study. Although only for the SDMT, time effects were observed in both groups accompanied by no group or interaction effect underlying this hypothesis. Moreover, with regard to the subgroup analysis, no group (HIIT vs. CG) effect could be observed, which also indicates no superiority of one exercise regime with regard to cognitive performance. A recently published secondary analysis investigated the effects of a high-intensity aerobic exercise intervention compared to a waitlist control condition on cognitive performance in pwMS, thereby

TABLE 2 | ANCOVA results of the overall analysis (HIIT vs. CG).

	Descriptive analysis				ANCOVA	
	HIIT ($n = 38$)		CG ($n = 36$)		Time p-Value F-Value (df = 1) Partial η^2	Group*Time p-Value F-Value (df = 1) Partial η^2
	T0	T1	T0	T1		
SDMT (points)	43.44	47.11	42.63	46.43	0.040*	0.912
	(9.95)	(10.36)	(13.76)	(14.76)	4.367	0.012
					0.059	0.000
VLMT (points)	52.08	52.74	52.37	52.77	0.003*	0.914
(Total score trials 1–5)	(8.95)	(10.87)	(11.73)	(10.3)	9.522	0.012
					0.120	0.000
BVMT-R (points)	20.42	19.89	20.09	19.26	0.000*	0.718
	(7.4)	(6.55)	(7.95)	(7.25)	17.827	0.131
					0.203	0.002
Rel. VO2peak (mL kg^{-1} min^{-1})	19.04	21.58	19.25	20.84	0.001*	0.126
	(5.61)	(5.84)	(5.12)	(5.2)	11.101	2.401
					0.135	0.033
Rel. power output (watts/kg)	1.34	1.63	1.32	1.47	0.000*	0.011*
	(0.53)	(0.54)	(0.44)	(0.45)	19.077	6.869
					0.212	0.088
Fatigue (FSMC)	69.45	66.42	62.84	61.67	0.305	0.404
	(15.66)	(13.41)	(17.03)	(17.60)	1.066	0.704
					0.015	0.010

*Data are presented as mean (standard deviation). HIIT, high-intensity group; CG, control group; T0, baseline; T1, post-intervention; SDMT, Symbol Digit Modalities Test; VLMT, Verbal Learning Memory Test; BVMT-R, Brief Visuospatial Memory Test-Revised; rel., relative; FSMC, Fatigue Scale for Motor and Cognitive Functions. *Significant main effect (time) or interaction (time*group).*

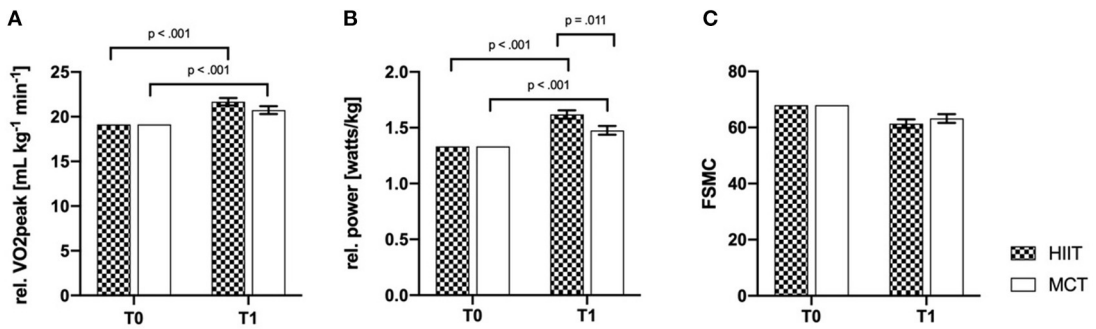

FIGURE 2 | Baseline-adjusted ANCOVA results for physical fitness outcomes **(A, B)** and fatigue **(C)** for the intervention (HIIT) and control group (CG). T0, baseline; T1, post-intervention; FSMC, fatigue scale for motor and cognitive functions; rel., relative. Deviation bars are shown as standard error.

analyzing effects on both the overall sample and a cognitive impaired subsample (29). Results show similar effects to the present analysis, as no interaction effects were observed for the overall analysis. However, based on between-group point estimates, the cognitive impaired subgroup showed clinically significant improvement in SDMT and similar improvements for the selective reminding test.

Besides potential exercise regime independent benefits of exercise on cognition, HIIT applied in this study had a positive impact on physical fitness in pwMS. Against argued worries about potential losses of adherence linked to higher exercise intensities (30), this study showed high adherence rates by pwMS of several disability ranges. However, the setting remains an inpatient rehabilitation that is not comparable to outpatient settings. Concerning the reached exercise intensities, it should be noted that the average HR of the CG was higher than prescribed. This could explain why time × group interaction for rel. VO2peak did not reach significance. Reasons for higher exercise intensities of the CG might emerge since individual exercise intensities were derived from baseline CPET. However, CPET was conducted until the participant's symptom reached maximum so that muscular fatigue, especially in

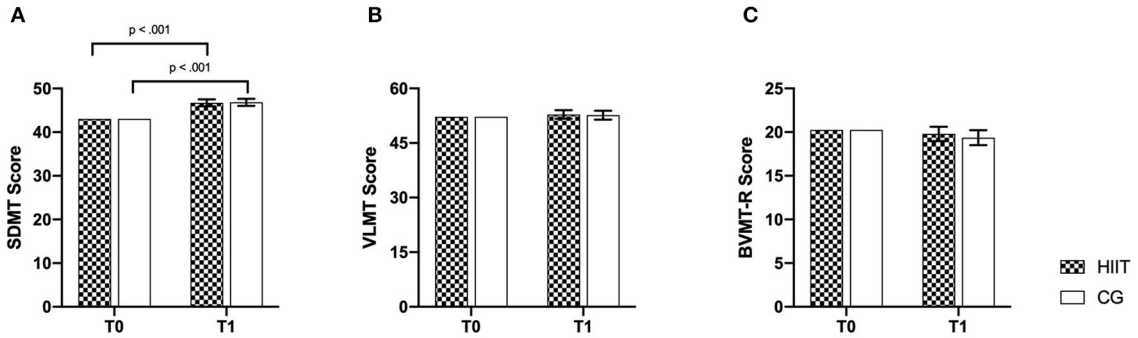

FIGURE 3 | Baseline-adjusted ANCOVA results for cognitive performance parameters for the intervention (HIIT) and control group (CG). T0, baseline; T1, post-intervention. **(A)** SDMT, Symbol Digit Modalities Test; **(B)** VLMT, Verbal Learning Memory Test; **(C)** BVMT-R, Brief Visuospatial Memory Test-Revised. Deviation bars are shown as standard error.

TABLE 3 | MANOVA results of the subgroup analysis (impaired cognition vs. intact cognition).

Descriptive analysis								
	Participants with impaired cognition				Participants with intact cognition			
	HIIT ($n = 15$)		CG ($n = 19$)		HIIT ($n = 23$)		CG ($n = 16$)	
	T0	T1	T0	T1	T0	T1	T0	T1
SDMT (points)	37.8 (8.41)	41.73 (9.69)	32.84 (7.23)	36.16 (8.35)	47.13 (9.25)	50.61 (9.38)	54.25 (10.04)	58.63 (10.86)
VLMT (points) (Total score trials 1–5)	48.6 (8.67)	51.4 (11.54)	47.37 (10.48)	50.37 (9.73)	54.35 (8.56)	53.61 (10.59)	58.31 (10.51)	55.63 (10.54)
BVMT-R (points)	14.93 (5.26)	17.27 (5.26)	15.89 (6.91)	15.74 (6.7)	24 (6.37)	21.61 (6.84)	25.06 (6.09)	23.44 (5.57)

MANOVA				ANOVA			
Group	Cognition	Group*Cognition		Group	Cognition	Group*Cognition	
p-Value *F*-Value (df = 3) Partial η^2	*p*-Value *F*-Value (df = 3) Partial η^2	*p*-Value *F*-Value (df = 3) Partial η^2		*p*-Value *F*-Value (df = 3) Partial η^2	*p*-Value *F*-Value (df = 3) Partial η^2	*p*-Value *F*-Value (df = 3) Partial η^2	
0.881	0.013	0.558	SDMT	0.905	0.791	0.512	
0.222	3.857	0.695		0.014	0.071	0.434	
0.010	0.147	0.030		0.000	0.001	0.006	
			VLMT	0.621	0.011	0.544	
			(Total score trials 1–5)	0.247	6.872	0.373	
				0.004	0.091	0.005	
			BVMT-R	0.525	0.025	0.232	
				0.408	5.263	1.457	
				0.006	0.071	0.021	

Data are presented as mean (standard deviation). HIIT, high-intensity group; CG, control group; T0, baseline; T1, post-intervention; SDMT, Symbol Digit Modalities Test; VLMT, Verbal Learning Memory Test; BVMT-R, Brief Visuospatial Memory Test-Revised.

pwMS with higher disability ranges, could occur prior to cardiovascular exhaustion.

This study has some limitations that need to be taken into account when interpreting the results. First, the study was a subgroup analysis, and the division into pwMS with impaired and intact cognition was done afterwards, consequently leaving the original randomization out. However, except for sex (in the subgroup analysis), no baseline differences were observed. Moreover, the sample size calculation was based on the primary outcome of the original trial. However, it is relatively large compared to existing trials in this research context. Second, the study was conducted during inpatient rehabilitation, enhancing adherence toward the exercise interventions but limiting the total time of the intervention period, since a normal stay at the clinic lasts 3 weeks. Consequently, potential neuronal adaptations may not fully develop in that relatively short period of time. Considering the inpatient rehabilitation setting, a passive or waitlist CG was due to ethical reasons not possible to establish. Moreover, it cannot be ruled out that other therapies within the clinical stay may have an impact on the changes

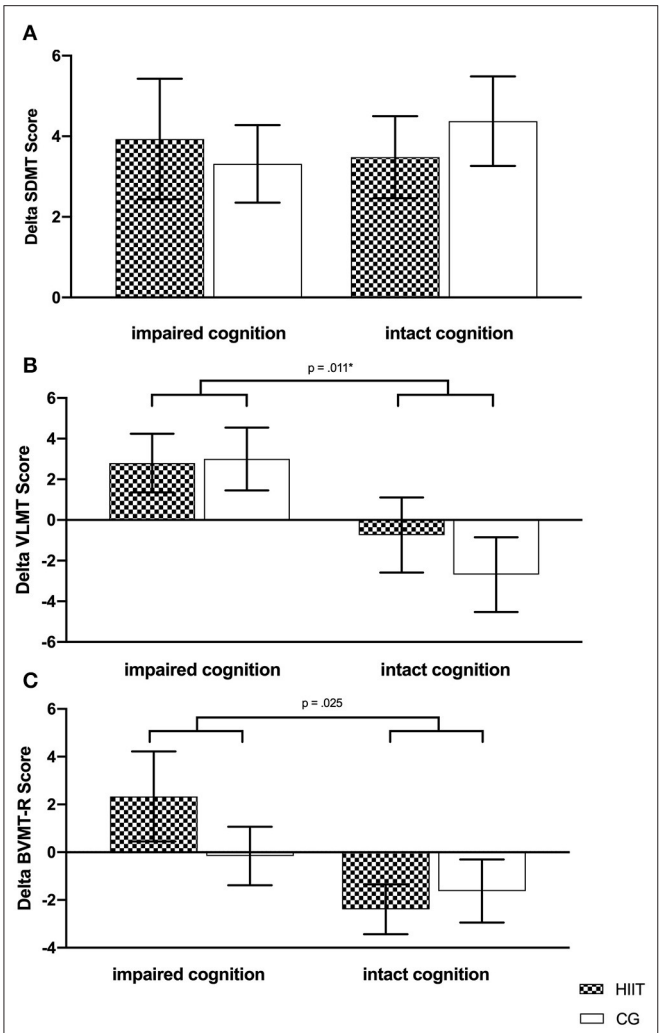

FIGURE 4 | Changes of cognitive performance parameters based on baseline cognition for the intervention (HIIT) and control group (CG). **(A)** SDMT, Symbol Digit Modalities Test; **(B)** VLMT, Verbal Learning Memory Test; **(C)** BVMT-R, Brief Visuospatial Memory Test-Revised. Deviation bars are shown as standard error. *Significant changes between groups of cognitive status.

in cognitive performance of the participants, especially with impaired cognition, contributing to the observed time effects. Third, test batteries of cognitive performance, such as the BICAMS, might not detect changes of cognitive performance within this short period of time but rather function as an assessment tool to evaluate baseline cognitive function. Fourth, no habituation phase was applied; thus, it cannot be excluded that cognitive performance was biased by learning effects toward habituation of the testing procedures. Fifth, more sensitive methods [e.g., biomarker of neuronal damage, imaging (MRI)] supported by test batteries of cognitive performance potentially reveal more meaningful results. Sixth, since muscular fatigue might bias the results of CPET and derived exercise intensities, other less vulnerable methods should be considered in the future to define exercise intensity. Seventh, although we applied one of the most frequently used and recommended test batteries, we cannot exclude the fact that the results might be linked to ceiling effects. Finally, it should be noted that one intervention group consisted only of female participants since sex was no stratification factor during the randomization process.

Recently published protocols of large-scaled RCTs reveal promising insights into future investigations that consider the limitations of existing studies and function as an example for other upcoming research (31, 32). Moreover, the present subgroup analysis should be enlarged in the future by conducting a prospective RCT, including the same intervention types for both persons with impaired cognition and those with intact cognition.

In conclusion, this study supports the need of RCTs that include cognitive performance as a primary endpoint and define eligibility based on baseline cognitive performance (impaired cognition vs. intact cognition). Future investigations should also conduct a sample size calculation based on the primary outcome of cognitive performance and consider habituation phases and test paradigms that are sensitive enough to detect changes of cognitive performance in a limited period of time.

DATA AVAILABILITY STATEMENT

The raw data were generated at the Rehabilitation Clinic Valens. Derived data supporting the findings of this study are available from AR upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethikkommission Ostschweiz (EKOS): EKOS18/96; Project ID: 2018–01378 Scheibenackerstrasse 4, 9000 St. Gallen. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PZ, WB, JK, RG, and JB designed the study. AR, NJ, and SP conducted data acquisition. AR and PZ conducted statistical analysis. AR drafted the manuscript under supervision of PZ and JB. DL gave expert input. All authors revised and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.619500/full#supplementary-material

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Thalamic Injury and Cognition in Multiple Sclerosis

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Multiple sclerosis (MS) produces demyelination and degeneration in both gray and white matter. Both cortical and deep gray matter injury is observed during the course of MS. Among deep gray matter structures, the thalamus has received special attention, as it undergoes volume loss in different MS subtypes and is involved in the earliest form of the disease, radiologically isolated syndrome. The thalamus plays an important role as an information relay center, and involvement of the thalamus in MS has been associated with a variety of clinical manifestations in MS, including fatigue, movement disorders, pain, and cognitive impairment (CI). Similar to thalamic volume loss, CI is seen from the earliest stages of MS and is potentially one of the most debilitating manifestations of the disease. The thalamus, particularly the dorsomedial nucleus as part of the basolateral limbic circuit and anterior thalamic nuclei through connections with the prefrontal cortex, has been shown to be involved in CI. Specifically, several cognitive performance measures such as processing speed and memory correlate with thalamic volume. Thalamic atrophy is one of the most important predictors of CI in MS, and both thalamic volume, diffusion tensor imaging measures, and functional activation correlate with the degree of CI in MS. Although the exact mechanism of thalamic atrophy is not well-understood, it is hypothesized to be secondary to degeneration following white matter injury resulting in secondary neurodegeneration and neuronal loss. The thalamus may represent an ideal biomarker for studies aiming to test neuroprotective or restorative therapies aimed at cognition.

Keywords: cognitive impairment, multiple sclerosis (MS), MRI, neurodegeneration, thalamus

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INTRODUCTION

Multiple sclerosis (MS) is one of the most common causes of neurological disability in young people, and cognitive impairment (CI) can be seen in 40–60% of patients (1, 2). CI can be seen in all causes of the disease, and MS commonly affects patients during peak years of productivity; thus, CI can lead to a significant burden for patients and society (1, 2). CI occurs despite a few patients with MS progressing to full-blown dementia (3). CI in patients with MS has been associated with reduced rates of employment, affecting the quality of life, independence, social participation, income, and access to health care (4, 5).

Although all cognitive domains may be affected in MS, the most commonly affected domains include learning and memory, speed of information processing, attention, and executive functioning, language, and social cognition (6–8). In patients with MS, predicting the severity of CI can be difficult. Some of the predictors for severity of CI in MS include greater age, disability score, cognitive reserve, sex, and possibly genetic markers (9, 10).

Lesion volume of demyelinated lesions in MS usually demonstrates a limited correlation with CI and cannot be used to accurately predict CI severity (11). Several other mechanisms regarding CI in MS include impairment of gray matter networks, atrophy of cortical and deep gray matter structures, including the thalamus (12, 13).

The thalamus has been implicated in CI in MS and several other neurological disorders. Reduced thalamic volume has been associated with CI in patients with epilepsy (14), dementia (15), stroke (16), and traumatic brain injury (17). In this article, we will review the role of the thalamus in cognition in MS.

THALAMUS AND COGNITION

Anatomically, the thalamus is composed of several different nuclei groups, including a lateral nuclear group, a medial nuclear group, anterior nuclear group, midline thalamic nuclei, reticular nucleus, and intralaminar nuclei (18). Functionally, the thalamus is classically divided into three groups, including the principal (relay) nuclei, association nuclei, and midline and intralaminar nuclei (19). Due to its integral function as a relay and integration center and taking part in several thalamocortical circuits, the role of the thalamus in cognition is well-recognized not only as a passive triage center but also contributing cognitive processes, including attention, speed of information processing, and memory (20).

The pulvinar nuclei are part of the lateral nuclei group and account for ∼25% of the thalamic mass (21). Pulvinar nuclei have been shown to play a role in selective attention, as evident by increased glucose uptake during positron emission tomographic imaging (22). The anterior thalamic nucleus, through its connections to the hippocampus *via* the fornix and mammillothalamic tract, is associated with encoding content and contextual information and recollective processes (23, 24). The medial dorsal thalamic nucleus, with its connections to the prefrontal cortex and the limbic system through the basolateral limbic system, is related to executive aspects of memory, including strategic memory retrieval of information to be remembered and familiarity processes (23, 24). The intralaminar/midline thalamic nuclei, through their connections to the parietal lobe, play a role in attention, arousal, awareness, and activation of cortical regions necessary for the processing of information to be stored (23, 24). Disruption of thalamocortical white matter tracts has been shown to inversely correlate with cognitive domains such as verbal memory (25).

THALAMUS AND MULTIPLE SCLEROSIS

MS is a progressive inflammatory and neurodegenerative disease of the human CNS that leads to demyelination and neuronal/axonal loss, and both cortical and subcortical demyelination are observed during the course of MS, including gray matter structures such as the thalamus, hippocampus, caudate, putamen, globus pallidus, and other structures of the basal ganglia (26, 27).

Imaging of the Thalamus in Multiple Sclerosis

T1- and T2-weighted images are less sensitive to detect lesions in gray matter regions due to inherent structural differences between gray matter and white matter and different inflammatory responses in those compartments (28–31). Thalamic lesions are usually more visible than cortical lesions, likely due to a higher density of myelin in the thalamus (32).

Thalamic lesions occur in two main types, subependymal or perivascular, and are present in 42–97% of patients (33–35). Imaging investigation in the thalamus have focused mainly on volumetric assessment (e.g., T1- or T2-weighted images, ultra-high field) and overall measures of thalamic integrity [e.g., susceptibility-weighted imaging (SWI), magnetization transfer ratio (MTR), magnetic resonance spectroscopy, diffusion tensor imaging (DTI)]. Common imaging modalities to detect thalamic pathology in MS are summarized in **Table 1**.

Different imaging modalities may be associated with different domains of CI. For example, in one study, diffusivity changes were consistently associated with information processing speed [Symbol Digit Modalities Test (SDMT)] and visual memory (Brief Visuospatial Memory Test—Revised), and verbal memory (California Verbal Learning Test, second edition,) but magnetic susceptibility was related only to SDMT performance (41).

Given the complex structural units within the thalamus, there has been growing interest in the evaluation of thalamic subnuclei and subregions. Subregion analysis of the thalamus has demonstrated an association of CI with mean diffusivity of a dorsomedial nucleus, orbitofrontothalamic tract, and amygdalothalamic tracts (42). In a recent study evaluating the association of thalamic nuclei volumes and cognitive performance, the volumes of anterior, medial, lateral, posterior, and ventral thalamic nuclei correlated positively with SDMT, whereas Brief Visuospatial Memory Test—Revised correlated with volumes of anterior, lateral, and medial nuclei (43). In another study, SDMT was correlated with superior and anterior volumes of the thalamus (44). Given the broad connections of the thalamus, the subregional analysis of different thalamic connections has also been evaluated. Reduced information processing speed was associated with atrophy of the bilateral frontal connected subregions, whereas SDMT negatively correlated with atrophy of frontal, motor, and connected temporal subregions (45). Functional MRI studies in MS have shown varying results. In one study, MS patients with cognitive impairment had increased resting-state functional connectivity between frontal, motor, occipital, and temporal subregions and hippocampus, parahippocampal gyrus, and superior temporal cortex (46). In another study, SDMT, paced auditory serial addition test, and California Verbal Learning Test all correlated with resting-state functional connectivity of several thalamic connections in healthy controls, but this association was not observed in MS patients (47).

TABLE 1 | Different MRI modalities and their use in the evaluation of thalamus and cognitive domains.

Cognitive domain	Thalamic volume	DTI	SWI	MRS	UHF
Verbal memory	Negative correlation with CVLT2 (36, 37)	Negative MD correlation with CVLT2 (36) Negative FA correlation (38)			
Visuospatial memory	Negative correlation with BVMTR (36, 37)	Negative MD correlation with BVMTR (36)	Negative pulvinar MP-LPV correlation with BVMTR (37)	Positive glutamate concentration correlation with PAL (39)	Negative thalamic volume and myelin density correlation with BVMTR (40)
Executive function (including attention and psychomotor speed)	Negative correlation with SDMT and PASAT (36, 37) Negative correlation with DKEFS (36, 37)	Negative MD correlation with SDMT, DKEFS, and PASAT (36) Negative FA correlation (38)	Negative pulvinar MP-LPV correlation with SDMT and DKEFS (37)		Negative thalamic lesion volume correlation with SDMT (34) Negative thalamic volume and myelin density correlation with DKEFS (40)

UHF, ultra-high field; SWI, susceptibility-weighted imaging; DTI, diffusion tensor imaging; MRS, magnetic resonance spectroscopy.

Thalamic Pathology

Clinically, thalamic involvement in MS manifests with a spectrum of diverse abnormalities, which range from fatigue and movement disorders to pain syndromes and cognitive decline. Given the broad range of the function of the thalamus, as described earlier, it is not surprising that thalamic involvement will have a wide variety of clinical manifestations in MS. Although the majority of evidence points to the thalamus as a site of secondary degeneration from distant white matter lesions, focal pathology within the thalamus may also, to some extent, affect function. Thalamic lesions have been found in around 71% of MS patients when imaged at 7 T (34, 35). The two main types of thalamic demyelinating lesions in MS include ovoid perivascular (perhaps due to extravasation of peripheral immune system cells from vasculature) and thin subependymal (perhaps due to diffusion of soluble toxin and chemokine infiltration from the CSF) lesions (33). In addition, histologically, dystrophic neurons, particularly in subependymal lesions (potentially excitotoxic injury), are observed, and the thalamic lesions contain a mixture of chronic active and inactive inflammation with or without demyelination (33).

Thalamic volume is inversely correlated with a physical disability and cognitive impairment in MS (33). There are likely several mechanisms for thalamic volume loss in MS. Progressive atrophy of the thalamus has been shown in all MS disease types (48), and loss of volume in the thalamus is one of the earliest and most prominent signs of deep gray matter pathology in patients with MS as seen in patients presenting with the clinically isolated syndrome (CIS) (49, 50) and radiologically isolated syndrome (51, 52). In another study, thalamic volume showed a weak relationship to physical disability score but was strongly correlated with cognitive performance in patients with MS (53). In a study evaluating the neuronal injury in the MS thalamus, authors found that both the thalamic volume and the concentrations of N-acetyl aspartate were decreased when compared with healthy controls (54). Thalamic neuronal density loss in MS (particularly smaller neurons) has been shown to occur at a faster rate compared with thalamic volume loss (30, 33). Further, the thalamic volume does not correlate with thalamic lesion volume, potentially implying that in addition to local inflammation, the volume loss could result from secondary neurodegeneration associated with the projecting tracts (30, 33). It should be noted that these studies were done at 3 T, and this association needs to be further studied using ultra-high field, where lesional pathology is more sensitively visualized. Retrograde degeneration can follow from focal white matter lesions in MS. The result of degeneration along white matter tracts could cause a neuronal loss in both cortical and deep gray matter structures, although this may not be the primary driving process in thalamic atrophy (55, 56). The impact of primary thalamic demyelination can potentially be better evaluated using MTR. MTR is a proposed marker of myelin content, and given the mixed nature of the thalamus as a white and gray matter, a structure may be more sensitive to this technique than other deep gray matter structures. MTR is decreased in the normal-appearing gray matter early in the disease course (mean duration of 1.9 years) and with mild clinical impairments (57).

DTI can detect changes compared with healthy controls in the normal-appearing thalamus, and the degree of thalamic changes correlate with functional impairment (31). White matter fractional anisotropy in the thalamus has been strongly associated with cognitive performance in MS patients (38). A DTI study examining thalamic connectivity using a stepwise regression analysis showed that thalamocortical lesion volume and the mean diffusivity in tracts connecting lesion and thalami were significantly correlated with thalamic volumes, which is a finding not observed in regions outside the thalamocortical white matter (50). In patients with primary progressive MS, thalamic volumetry and DTI measures have been shown to correlate with the extent of T2 hyperintense lesions as well as with the severity of microscopic damage to the normal-appearing white matter and normal-appearing gray matter, suggesting that both local inflammatory demyelination, as well as changes secondary to axonal transection of fibers passing through areas of diseased brain white matter, can account for thalamic abnormalities atrophy (58). DTI measures can also be used to improve the segmentation of the thalamus, which can allow a better evaluation of thalamic subnuclei in future studies (59). The

role of iron in thalamic pathology is complex, as there have been mixed reports of iron content and concentration in MS, potentially due to different imaging techniques. In patients with MS, there is higher iron content compared with healthy controls (60), and increased thalamic susceptibility (proposed as iron content) using 7-T MRI has been seen in MS patients compared with healthy controls and associated with higher disability scores (61). This deposition may occur secondary to myelin and oligodendrocyte debris, as well as iron stores in macrophages, or it may be the product of hemorrhages from damaged brain vessels (62). Iron accumulation, particularly in the pulvinar, appears to be increased early in the course of the disease and reduced later in the course of the disease (63). Deposition of iron, as estimated by SWI, in the pulvinar nucleus of the thalamus has been shown to occur in the absence of volume change and atrophy, suggesting that this pathology may precede structure-specific atrophy (64). Increased iron accumulation in the early course of the disease can be potentially related to blood–brain translocation of heme-iron and microglial iron accumulation from higher perfusion supplied to thalamus compared with white matter, whereas the decrease in iron later in the course of the disease could potentially be related to reduction of oligodendrocytes density in thalamus (63). An alternative hypothesis suggests that increased iron concentrations may be the result of volume loss with a relatively fixed iron content, given the false impression of increased concentration (65).

LONGITUDINAL THALAMIC CHANGES

The majority of the information regarding the role of thalamic injury in the MS disease process has been demonstrated using thalamic volume. A longitudinal study evaluating thalamic volumes in patients with primary progressive MS showed volume loss of thalami at baseline in MS patients compared with healthy control and further loss of volume after 1 year (66). A significantly reduced thalamic volume at baseline has also been associated with the development of CI in MS patients at 2-years follow-up (55). There is also evidence that the thalamus loses volumes at a different rate through the disease span. Compared with CIS, patients with relapsing and remitting MS display a faster rate of thalamic atrophy (67). In addition, a lower burden of white matter lesions and higher lifetime cumulative exposure to disease-modifying therapy have been shown to correlate with a slower rate of thalamic atrophy (67). Interestingly, this same group found that the thalamus had the most volume change from age 30–60 years but, after 60 years, showed a greater contribution from normal aging (68). This was not true in the caudate and putamen, further suggesting lesion accumulation drives thalamic volume loss.

When MS patients were followed for 15 months, thalamic DTI changes were found to be independent predictors of disability score deterioration. After 15 months, there was an increase in thalamic mean diffusivity and a decrease in thalamic fractional anisotropy (58). At 5-years follow-up, a reduction in fractional anisotropy of the anterior thalamic radiation can be used to predict CI in MS patients (69).

DISCUSSION

Thalamic involvement is an important feature of MS and can be seen early in CIS and radiologically isolated syndrome. Thalamus is a central relay structure with multiple connections and underpins a broad range of functions. As such, the involvement of the thalamus in MS can be associated with several neurological deficits, including CI. This involvement can be due to a combination of white matter and gray matter pathology as a result of direct inflammatory and cytotoxic damage as well as indirect neurodegeneration secondary to damage to its widespread afferent and efferent tracts. The histopathological hallmarks include neuronal loss, active and chronic inflammation with or without demyelination, and accumulation of iron deposits. Traditional MRI modalities may not be sensitive to detect thalamic changes, but several novel MRI modalities, including SWI, functional MRI, DTI, and magnetic resonance spectroscopy, have been used to evaluate thalamic pathology and their correlation with CI. With our improved understanding of the complex structure and connections within the thalamus, there is a need for better evaluation of these anatomical and connectivity subregions. Although there have been some recent advances in this area, there are limitations in spatial resolution, which could improve with advances in imaging techniques. There is also a paucity of research in histopathological changes in different thalamic subregions, which could be crucial in advancing our understanding of the pathophysiology of thalamic pathology. Furthermore, the thalamic pathology is an ongoing process throughout the course of the disease, although its rate can be affected by the activity of the disease. Many of the studies evaluating CI in MS are cross-sectional, and there are only limited reports studying the longitudinal changes in the thalamus and its subnuclei. There is a role for further research in understanding thalamic pathology, its progression, prevention, and targets for potential therapies for neurological restoration to prevent, delay, and reverse the impact of CI in MS patients.

AUTHOR CONTRIBUTIONS

MA and DO contributed to the design, literature review, analysis, and writing of the manuscript. All authors contributed to the article and approved the submitted version.

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An Interview-Based Assessment of the Experience of Cognitive Impairment in Multiple Sclerosis: The Cognitive Assessment Interview (CAI)

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Background: Cognitive impairment is a common feature of multiple sclerosis (MS). A semi-structured interview, including informant input, can characterize the experience of individuals living with MS and cognitive involvement.

Objective: We administered the Cognitive Assessment Interview (CAI), a patient- and informant-based semi-structured interview, to characterize the experience of cognitive impairments in those living with MS.

Methods: Trained raters administered the CAI to a sample of MS participants and their informants enrolled for a trial of cognitive remediation. Cognitive impairments on the CAI were characterized and compared to those captured by neuropsychological and self-report measures.

Results: A total of $n = 109$ MS participants (mean age $= 50.3 \pm 12.2$) and their available informants ($n = 71$) were interviewed. Participants reported experiencing processing speed (90/106, 85%), working memory (87/109, 80%), and learning and memory (79/109, 72%) problems most commonly. CAI-based ratings were moderately correlated with a self-report measure (Multiple Sclerosis Neuropsychological Screening Questionnaire, $r_s = 0.52$, $p < 0.001$) and only mildly correlated with objective neuropsychological measures specific to executive functions ($r_s = 0.21$, $p = 0.029$). For those with informant interviews, ratings were overall consistent, suggesting that the CAI is valid even in cases in which an informant is unavailable and the interview is conducted with the patient alone (as is often the case in clinical and research settings).

Conclusions: The CAI provides a semi-structured interview to characterize the experience of cognitive impairment in MS, with findings representing real-world functioning, adding valuable information to both self-report measures and neuropsychological assessment.

Keywords: multiple sclerosis, cognitive, neuropsychological, cognitive assessment interview, CAI, daily functioning

INTRODUCTION

Cognitive difficulties affect up to 70% of individuals living with multiple sclerosis (MS) (1, 2) and are associated with significant disability and overall reduction in quality of life (3, 4). Objective impairments are most commonly found in the domains of processing speed and efficiency, complex attention and working memory, and novel learning (1, 2, 5). A semi-structured interview, in which a patient's self-report is reviewed by the expert judgment of a skilled interviewer and corroborated with a report of a significant other who lives with the patient and observes them daily, can be an important measure for fully characterizing the impact of cognitive impairments on daily cognitive functioning.

Structured and semi-structured interviews are considered the gold standard accompaniment to objective cognitive measures in other disorders with cognitive involvement, and specifically for use in understanding the experience of age-related dementias, including the Clinical Dementia Rating (CDR) scale (6, 7), the Interview for Deterioration in Daily Living Activities in Dementia [IDDD; (8)], and the Disability Assessment for Dementia [DAD; (9)]. Items in these scales probe the patient's ability to complete activities of daily living, such as bathing, dressing, eating, attending community events, and using the telephone. However, the items in these scales are designed for those of older age and with more advanced forms of cognitive impairment, therefore resulting in a "ceiling effect" for many with MS, often missing the impact of milder and more subtle areas of impairment.

The Cognitive Assessment Interview [CAI; (10)] was developed to assess daily functioning in patients with schizophrenia, who in comparison to those with age-related dementias are typically of a younger age and have a higher level of cognitive functioning, with domains of involvement including working memory, attention, verbal learning and memory, reasoning and problem solving, speed of processing, and social cognition, that may be more applicable for those with MS. The CAI consists of 10 semi-structured interview questions and is conducted by a trained clinical rater with a patient, as well as with an informant (e.g., caregiver or spouse) when available. The interview ratings (provided by the clinical rater) are based on the patient's and informant's responses and examples. The CAI has demonstrated good test–retest and interrater reliability, high internal consistency, and significant correlation with functional and objective cognitive measures (10–13).

Several self-report measures of daily cognitive functioning were developed for use in MS, including the Multiple Sclerosis Neuropsychological Screening Questionnaire [MSNQ; (14, 15)] and the perceived deficits questionnaire [PDQ; (16)]. These questionnaires provide useful insight into a patient's perception of their cognitive difficulties. However, they may not accurately reflect the true level of cognitive impact on daily functioning, due to either over- or underestimation of subjective experiences and the influence of mood states in ratings [e.g., depression; (14–17)]. The CAI has been shown to be independent from depressive symptoms (11), suggesting that raters were effectively able to differentiate their participants' cognitive complaints from mood symptoms.

The aim of the current study was to utilize the CAI to characterize the experience of cognitive impairment for people living with MS. Given its rating of relevant cognitive domains (e.g., processing speed and working memory), suitability for use in younger and higher functioning patients, and utilization of informant input, we hypothesize that the CAI is suitable for additional characterization of impairment, separate from objective neuropsychological and self-report measures. To test our hypothesis, we administered the CAI to a large sample of people with MS reporting cognitive difficulties, as well as to their informants where possible, and characterized daily cognitive impairment in MS based on its findings. We additionally compared CAI findings to performance on a battery of neuropsychological measures sensitive to MS-related cognitive impairment, as well as an objective measure [the Test of Everyday Cognitive Ability (TECA); (18)] and a self-report measure of daily cognitive functioning (i.e., MSNQ).

METHODS

Participants

Participants were enrolled in a clinical trial of a cognitive remediation program (19). All participants had a confirmed diagnosis of MS [all subtypes were included; (20)] and had at least mild cognitive impairment as defined by an age-normative z score of −1.0 or lower on the symbol digit modalities test (SDMT). Participants were also required to have an estimated premorbid functioning in the normal range, based on a standard score of 80 or above on a reading recognition test [the Wide Range Achievement Test, third edition; WRAT-3; (21)] as a proxy for premorbid intellectual functioning (22). Exclusion criteria included a history of developmental disorders, conditions other than MS that may cause cognitive impairment, a primary psychiatric disorder, a substance use disorder, any other major medical disorder, and relapse or steroid use in the month prior to enrollment.

All participants provided written informed consent to study procedures that were approved by the Institutional Review Board and the Committee on Research Involving Human Subjects at Stony Brook Medicine, Stony Brook, New York, and in compliance with the Helsinki Declaration.

Cognitive Assessment Interview

Two clinical raters completed standardized training for CAI administration (provided by Dr. Ventura) and met consensus for rating of example recorded video interviews. The CAI consists of 10 items addressing six cognitive domains (**Table 1**): Attention/Concentration, Working Memory, Verbal Learning and Memory, Reasoning and Problem Solving, Speed of Processing, and Social Cognition. Raters interviewed participants and available informants separately. Informants were defined as someone identified by the participant who lives with them and is familiar enough to comment on their cognitive and daily functioning (spouse, partner, caregiver, or adult child).

TABLE 1 | Cognitive Assessment Interview (CAI) summary.

CAI item	Cognitive domain
Item 1: Difficulty maintaining newly learned information in mind for brief periods (long enough to use)?	Working memory
Item 2: Difficulty performing "on the spot" mental manipulations or computations?	
Item 3: Problems sustaining concentration over time (without distraction)?	Attention/ Concentration
Item 4: Difficulty focusing on select information (if there is not obvious distraction)?	
Item 5: Trouble learning and remembering verbal material?	Verbal learning and memory
Item 6: Difficulty recalling recent events?	
Item 7: Lack of flexibility in generating alternate plans when needed?	Reasoning and problem solving
Item 8: Problems in situations requiring judgment?	
Item 9: Performs tasks slowly?	Speed of processing
Item 10: Difficulty appreciating another person's intentions/point of view?	Social cognition
Global severity score	Global cognitive functioning
Global assessment of functioning score	

TABLE 2 | Summary of neuropsychological measures and Cognitive Assessment Interview (CAI) items for each of the cognitive domains on the CAI.

Cognitive domain	Neuropsychological measures	CAI items
Working Memory	Wechsler Adult Intelligence Scale, 4th Edition (WAIS-IV)[a]: Digit Span Backward and Letter–Number Sequencing subtests	1, 2
Attention	WAIS-IV[a]: Digit Span Forward	3, 4
Learning and Memory	Selective Reminding Test (SRT)[b] Brief Visuospatial Memory Test—Revised (BVMT-R)[c]	5, 6
Executive Functioning	Delis-Kaplan Executive Function System (D-KEFS)[d]: Trail Making Test, Alternating Numbers and Letters Condition	7, 8
Processing Speed	Paced Auditory Serial Addition Test (PASAT): 2 s condition[e]	9
Social Cognition	Symbol Digit Modalities Test (SDMT)[f]	10

[a]Wechsler (23); Drozdick et al. (24); [b]Buschke (25); [c]Benedict and Groninger (26); [d]Delis et al. (27); [e]Diehr et al. (28); [f]Smith (29).

Each domain is rated according to the presence and severity of impairment based on the participant and informant (when available) responses to semi-structured questions and prompts for examples of cognitive difficulties from 1 (no impairment) to 7 (severe impairment). In addition, the clinical rater provides an overall global severity (GS) score of cognitive impairment, from 1 to 7, and global assessment of functioning cognition ratings (GAF-Cog) ranging from 0 to 100 (with 0 being most severely impaired and 100 being the most highly functional).

Cognitive Measures

Serving as the baseline evaluation for the clinical trial, participants completed an objective battery of tests addressing cognitive domains similar to those addressed with the CAI, including working memory, attention, learning and memory, executive functions, and processing speed (see **Table 2** for summary of tests and domains). Briefly, the Digit Span Backward condition (Digit Span subtest) and the Letter–Number Sequencing subtest from the Wechsler Adult Intelligence Scale, 4th Edition [WAIS-IV; (23)] were used as measures of working memory. The WAIS-IV Digit Span Forward condition from the Digit Span subtest was used as a measure of attention. Verbal learning was assessed with the learning trials on the Selective Reminding Test [SRT; (25)], and visual learning was assessed with the learning trials on the Brief Visuospatial Memory Test—Revised [BVMT-R; (26)]. The Trail Making Test, Alternating Numbers and Letters condition from the Delis-Kaplan Executive Function System [D-KEFS; (27)] was used to assess executive functions. Finally, the Paced Auditory Serial Addition Test [PASAT; (28)], 2 seconds condition, and the SDMT measured information processing speed. While no objective test of social cognition was administered, measures of complex information processing speed (i.e., PASAT and SDMT) were also used to compare to the social cognition domain on the CAI, based on previous studies demonstrating a strong link between these cognitive functions (30).

For all cognitive measures, raw scores of each participant were converted into age-normed *z* scores. In cases where two measures were used to assess one cognitive domain (i.e., working memory, learning and memory, and processing speed), *z* scores from both tests were averaged into one domain score. Normative *z* scores were also averaged across all domains to obtain a composite *z* score to serve as a measure of global cognitive functioning.

As an objective measure of real-world functioning, the TECA (18) was also administered. The TECA is a 10-item test of timed instrumental activities of daily living (e.g., reading a grocery list, counting change) developed for use in MS. For a subjective measure of functioning, participants completed the MSNQ, a self-report measure of daily cognitive functioning (14).

Statistical Analysis

We first characterized the sample based on CAI findings. Mean and standard deviations were computed for the sample's demographic and clinical characteristics. To characterize daily cognitive impairments in our sample based on CAI ratings, means, standard deviations and frequencies of global cognitive impairment, as well as impairment within the CAI domains were calculated. Finally, given that in MS, not all participants will have an available informant for interview, we also tested the consistency of ratings between the participant and informant interviews. Thus, intraclass correlation coefficient (ICC) estimates and their 95% confident intervals were calculated between participant-based and informant-based ratings to better capture the relationship between the two.

TABLE 3 | Demographic and clinical features of the sample.

Patients (n = 109)	
Mean age (range)	50.3 (18–69)
Mean years education (range)	14.8 (11–20)
Percent female	78%
Race *n* (%)	
Caucasian	92 (84.4%)
African-American	8 (7.3%)
Unspecified	7 (6.4%)
Diagnosis *n* (%)	
Relapsing remitting MS	70 (64.2%)
Secondary progressive MS	28 (25.7%)
Primary progressive MS	6 (5.5%)
Not reported	5 (4.6%)
Median EDSS[a] (range)	3.5 (0–8.5)
Mean WRAT[b] standard score (range)	103.6 (80–119)
Mean SDMT[c] z score (SD)	−2.10 ± 0.99

[a] *The expanded disability status scale;* [b] *The wide range achievement test;* [c] *The symbol digit modalities test.*

To further examine the additive value of the CAI to traditional objective and self-report measures, we compared impairment, as measured by the CAI, to that identified by the neuropsychological measures, the TECA, and the MSNQ. As the CAI is measured on ordinal scales, data do not meet the assumptions for parametric statistics. Thus, non-parametric Spearman rank correlations were calculated between (1) global CAI and neuropsychological measures (including the TECA), (2) specific cognitive domains on the CAI and neuropsychological tests, and (3) global CAI indices and the MSNQ. All statistical analyses were performed using SPSS statistical package version 25.0 (31).

RESULTS

A total of n = 109 individuals with MS were interviewed using the CAI and assessed with an objective neurocognitive test battery. The majority of CAIs also included an informant (n = 71). Participants ranged in age from 18 to 69 (mean = 50.3 ± 12.2) years, were 78% female, and included those with relapsing remitting (64.2%) and progressive (31.2%) subtypes. See **Table 3** for full demographic and clinical characteristics of the sample.

CAI Ratings of the Experience of Cognitive Impairment in Daily Life

Global Impairment

Global severity ratings indicated at least minimal impairment (defined as a score of 2 or greater) in 92% of the sample (95/103, mean rating = 2.78 ± 1.0). Global assessment of functioning was at least mildly impaired in 50% of the sample (defined as rating score ≤ 70; mean rating = 74.0 ± 14.0), including 29% with mild impairment (GAF-Cog = 61–70), 19% with moderate impairment (GAF-Cog = 51–60), and 2% with severe impairment (GAF-Cog ≤ 50).

Frequency and Severity of Impairment Across Domains

The domains with the highest percentage of impairment (scored as 2 or greater) were Speed of Processing (90/106, 85%, mean rating = 2.78 ± 1.12), followed by Working Memory (87/109, 80%, mean rating = 2.51 ± 0.9), Verbal Learning and Memory (79/109, 73%, mean rating = 2.42 ± 0.9), and Attention/Concentration (64/109, 59%, mean rating = 2.19 ± 0.99), while the domains of Reasoning and Problem Solving and Social Cognition were less affected in our sample (28 and 37%, respectively) (**Figure 1**, **Table 4**). Among those individuals with any impairment (rated >1), the overall severity level was rated as mild (mean = 2.57 ± 0.83).

Consistency of Ratings Between Participant and Informant Interviews

Among those participants who also had informant interviews (n = 71), intraclass correlations were calculated between participant-based and informant-based ratings. ICC estimates and their 95% confident intervals were calculated based on an average-rating, absolute-agreement, 2-way random-effects model. The ICC between participant-based and informant-based ratings was fair [ICC = 0.67, 95% CI = 0.62 to 0.72, $F_{(628,628)}$ = 3.10, $p < 0.001$]. On average, participant-based ratings indicated higher severity on daily tasks requiring working memory (mean rating = 3.14 ± 1.17), verbal learning and memory (mean rating = 2.58 ± 1.25), and information processing speed (mean rating = 2.74 ±1.28), while informant-based ratings were in the minimal range of severity in these domains. Interestingly, informant-based ratings indicated, on average, some difficulty in cognitive flexibility (mean rating = 1.59 ± 0.97) and social cognition (mean rating = 1.65 ± 1.14), while participant ratings indicated, on average, no impairment in these domains.

Correspondence Between CAI and Objective Neuropsychological Assessment Measures

To test correspondence between the CAI and objective neuropsychological measures on global cognitive functioning, Spearman's rank-order correlation analysis was performed between the CAI global indices (GS and GAF-Cog) and the neuropsychological global measures (composite z score and TECA). While TECA and composite z scores significantly correlated with each other ($r_s = -0.58$, $p < 0.001$), no significant correlations were identified between the GS or GAF-Cog and composite z scores (GS $r_s = -0.13$, $p = 0.195$; GAF-Cog r_s = 0.17, $p = 0.082$). Similarly, there were no significant correlations between GS or GAF-Cog and the TECA (GS $r_s = 0.05$, $p = 0.646$; GAF-Cog $r_s = -0.13$, $p = 0.186$).

To test correspondence between the CAI cognitive domains and objective neuropsychological domains, a Spearman's rank-order correlation analysis was performed between ratings of CAI individual cognitive domains and the participants' performance

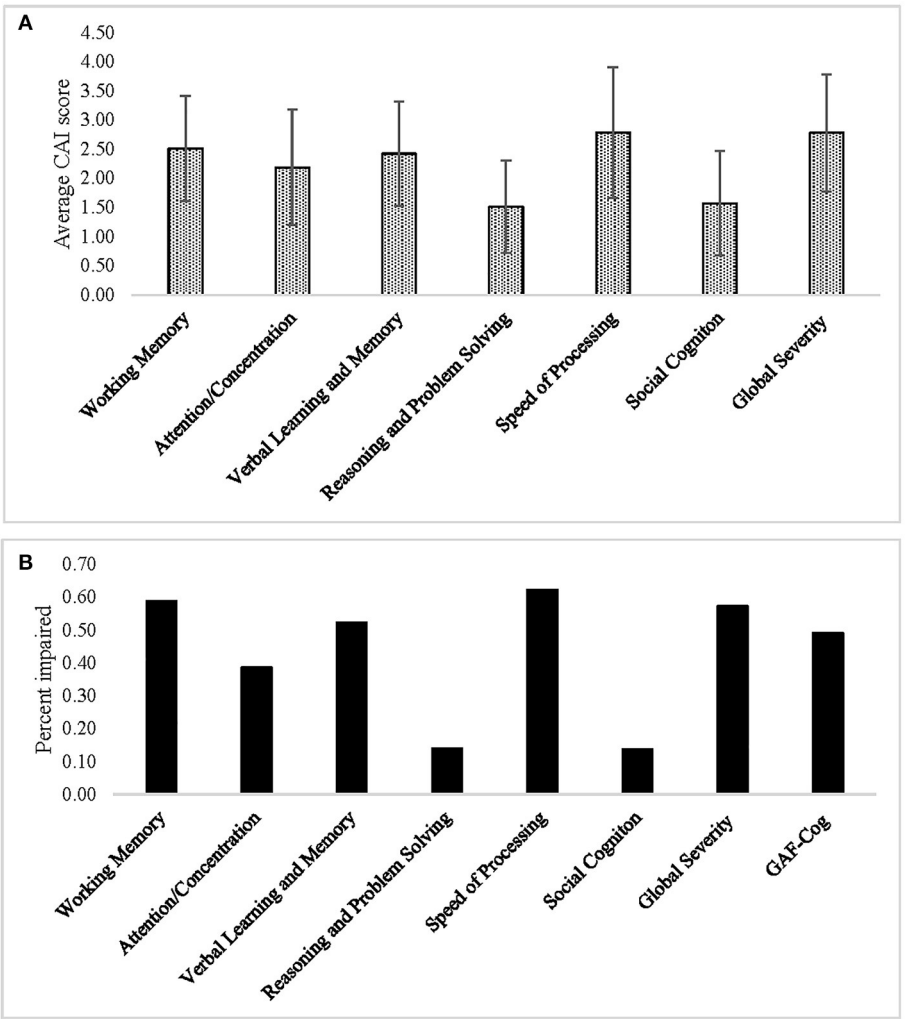

FIGURE 1 | (A) Average ratings and **(B)** percent impairment for each of the Cognitive Assessment Interview (CAI) cognitive domains, as well as for the Global Severity and Global Assessment of Functioning (GAF-Cog) scales.

on the corresponding objective neuropsychological domains (**Table 2**). Results indicated a mild correlation between CAI ratings and the participant's cognitive performance in the domain of executive functions (e.g., reasoning and problem solving; $r_s = -0.21$, $p = 0.033$). Executive function performance was also mildly correlated with the GAF-Cog ($r_s = -0.21$, $p = 0.029$). No other statistically significant correlations were identified between the CAI and neuropsychological measures for any of the other domains.

Correspondence Between CAI and MSNQ

Spearman's rank-order correlation analysis was performed between the CAI global scores (GS and GAF-Cog scales) and the MSNQ, a self-report questionnaire assessing daily cognitive functioning ($n = 103$). Moderate correlations were identified between the MSNQ and patient ratings on both global indices of the CAI (GS $r_s = 0.52$, $p < 0.001$; GAF-Cog $r_s = -0.43$, $p < 0.001$). The MSNQ did not significantly correlate with either the composite cognitive z scores ($r_s = -0.09$, $p = 0.365$) or the TECA scores ($r_s = 0.08$, $p = 0.448$).

DISCUSSION

This is the first study to use a semi-structured interview and include informant input to characterize the experience of cognitive impairment in a large sample of individuals with MS. In this MS sample of individuals meeting objective (SDMT) criteria for at least mild cognitive impairment, the CAI also indicated an overall mild cognitive impairment. Consistent with the expected areas of cognitive difficulties in MS (1, 2, 5), ratings on the CAI indicated that the most frequent experience of cognitive impairments were in the areas of processing speed, working memory, and verbal learning and memory (affecting more than 70% of the sample). Processing speed, specifically, was the leading area of difficulty, affecting 85% of our sample. It has

TABLE 4 | Frequency of impairment across the different Cognitive Assessment Interview (CAI) domains in the sample.

	Cognitive domain						
	Working memory	**Attention/ Concentration**	**Verbal learning and memory**	**Reasoning and problem solving**	**Speed of processing**	**Social cognition**	**Global severity score**
No impairment (1–1.5)	20.2% (*n* = 22)	41.3% (*n* = 45)	27.5% (*n* = 30)	72% (*n* = 77)	15.1% (*n* = 16)	63.3% (*n* = 69)	7.8% (*n* = 8)
Minimal (2–2.5)	45.9% (*n* = 50)	33.0% (*n* = 36)	35.8% (*n* = 39)	23.4% (*n* = 25)	22.6% (*n* = 24)	22.9% (*n* = 25)	35.0% (*n* = 36)
Mild (3–3.5)	22.0% (*n* = 24)	14.7% (*n* = 16)	32.1% (*n* = 35)	3.7% (*n* = 4)	38.7% (*n* = 41)	8.3% (*n* = 9)	34.0% (*n* = 35)
Moderate (4–4.5)	11.0% (*n* = 12)	10.1% (*n* = 11)	3.7% (*n* = 4)	0.9% (*n* = 1)	16.0% (*n* = 17)	4.6% (*n* = 5)	18.4% (*n* = 19)
Severe (5–7)	0.9% (*n* = 1)	0.9% (*n* = 1)	0.9% (*n* = 1)	0% (*n* = 0)	7.5% (*n* = 8)	0.9% (*n* = 1)	4.9% (*n* = 5)
Total impairment	79.8% (*n* = 87)	58.7% (*n* = 64)	72.5% (*n* = 79)	28% (*n* = 30)	84.9% (*n* = 90)	36.7% (*n* = 40)	92.2% (*n* = 95)

been argued that impaired processing speed is among the earliest cognitive functions to be affected by MS (32) and is thought to be related to deterioration of white matter integrity, affecting signal transmission speed and efficiency within and between brain networks (33, 34). In addition, it has been proposed that slowed information processing speed underlies other MS-related cognitive impairments, including working memory and novel learning (35, 36). Our findings expand the existing literature, demonstrating that slowed processing speed is a main area of difficulty affecting daily functioning in a large majority of individuals with MS with cognitive involvement.

As not all MS participants have an available informant for interview (e.g., those who live alone), we evaluated the contribution of the informant interview in the ratings of the MS patients. For the sample subset with informant interviews, ratings were overall consistent, suggesting that the CAI is valid even in cases in which informant is unavailable and interview is conducted with the patient alone (as is often the case in clinical and research settings). However, participant-based ratings indicated elevated levels of impairment on items assessing working memory, learning and memory, and information processing speed, compared to informant-based ratings. These findings correspond to the description of cognitive involvement in MS as an "invisible" or "hidden" symptom of the disease (37, 38), with patients often expressing that even the people who are closest to them (i.e., caregivers) underestimate the extent to which cognitive difficulties can affect their everyday functioning and quality of life. Conversely, informant-based ratings indicated some difficulty in reasoning and problem solving and in social cognition, while participant-based ratings indicated no impairment in these domains, suggesting that these are more "visible" cognitive manifestations that are more readily apparent to the patient's environment. Indeed, unlike working memory, learning and memory, and processing speed, these functions involve others to a greater extent. It is possible that the reduced self-awareness of patients to these cognitive changes may stem from an attribution error, as these difficulties are easier to attribute to the external environment, rather than to the self.

Across neuropsychological testing domains, executive functioning performances had the strongest correspondence to CAI findings and therefore may be most predictive of the experience of day-to-day cognitive functioning. In addition, CAI findings were moderately correlated with the subjective self-report measure, indicating that the interview-based format can provide additional and fuller detail than captured by a self-reported rating. Together, our findings suggest that the CAI can provide a unique characterization of the patient's experience of cognitive difficulties that may be distinct from what is captured by objective neuropsychological assessments and self-report measures. While neuropsychological tests are "clean" measures of specific cognitive domains administered in well-controlled settings, and self-reports offer an entirely subjective experience of cognitive difficulties by the patient, the CAI uniquely offers a more objective assessment of the patient's daily cognitive difficulties. Our finding that the TECA was significantly correlated with the composite score of objective cognitive measures but not with the CAI global indices or the MSNQ exemplifies this idea by demonstrating that an objective measure of daily cognitive abilities in the quiet, controlled environment of the lab or clinic is more closely related to other objectively assessed cognitive abilities rather than the patient's individual experience of day-to-day cognitive functioning in the real-world environment.

While the CAI has demonstrated good test–retest reliability and high internal consistency when administered to a sample of individuals with schizophrenia, these psychometric properties were not measured in the current study and would be important to assess in future studies. Indeed, the current work aimed to characterize, rather than validate, the CAI in MS. Nevertheless, we believe that these core psychometric qualities of the CAI would not be inherently different in our MS sample due to important characteristics shared by the two samples, such as a

wide age range (including young adults), relatively subtle changes in cognitive functioning (e.g., as compared to neurodegenerative disorders), and similar cognitive domains affected by the two conditions (e.g., processing speed).

As depression and fatigue are common in MS and may affect daily cognitive functioning, one limitation of the current study is the lack of mood and fatigue measures. In addition, while the CAI has been shown to be independent from depressive symptoms in individuals with schizophrenia (11), it would be important to determine whether this finding extends to the MS population as well. Therefore, it would be essential to include these measures in future studies using the CAI to improve our understanding of the relationship between mood and fatigue and daily cognitive functioning in MS, as measured by the CAI.

CONCLUSION

The present study is the first to characterize the impact of cognitive impairments on daily living in MS based on detailed interviews with a large sample of patients and caregivers. MS participants with at least mild objective cognitive impairment have overall mild CAI cognitive impairment as well, with aspects of processing speed and working memory being the most widely affected. The CAI captures aspects of real-world functioning that are distinct from both a self-reported inventory and objective cognitive testing, thus enriching the global understanding of the impact cognitive impairment may have on daily living in MS.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board and the Committee on Research Involving Human Subjects at Stony Brook Medicine, Stony Brook, New York. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LC, LK, TE-S, PB, JV, KS, and MS wrote, reviewed, and edited the manuscript. LC, TE-S, PB, and MS wrote the original draft. TE-S and PB done visualization. LC done validation, supervision, and funding acquisition. LC, MS, and KS were responsible for resources, investigation, data curation, and project administration. LC and KS designed the methodology. TE-S, LC, and MS done the formal analysis. LC, LK, KS, and MS conceptualized the study. All authors contributed to the article and approved the submitted version.

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Effects of Transcranial Direct Current Stimulation on Cognition, Mood, Pain, and Fatigue in Multiple Sclerosis: A Systematic Review and Meta-Analysis

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Background: The study aimed to evaluate the effects of transcranial direct current stimulation (tDCS) on cognition, mood disturbance, pain, and fatigue in people with multiple sclerosis (PwMS).

Methods: A literature search was performed on articles published between January 1990 and May 2020 in Pubmed, Medline, and Web of Science using the following keywords and their abbreviation in combinations: multiple sclerosis and transcranial direct current stimulation. Mean effect size (ES) and 95% confidence interval were calculated for each domain of interest.

Results: Seventeen articles with a total of 383 PwMS were included in this analysis. For cognition, a strong effect size was found for the trial administering the Symbol Digit Modalities Test (ES: 1.15), whereas trials applying the Attention Network Test showed a negative effect size of −0.49. Moderate to strong effect sizes were observed for mood disturbance (mean ES: 0.92), pain (mean ES: 0.59), and fatigue (mean ES: 0.60). Further subgroup analyses for MS-related fatigue showed that both high and low intensities of stimulation lead to nearly the same degree of favorable effects. More pronounced effects were observed in studies administering the Fatigue Severity Scale compared with studies using other fatigue measures such as the Modified Fatigue Impact Scale.

Conclusion: These results provide preliminary evidence that tDCS has a favorable effect on cognitive processing speed, mood disturbance, pain, and fatigue in MS. However, the effects on cognition and fatigue vary based on the specific assessment used.

Keywords: cognition, mood, pain, fatigue, multiple sclerosis, transcranial direct current stimulation

INTRODUCTION

Multiple sclerosis (MS) is the most common non-traumatic cause of neurological disability in young adults, affecting ∼1,000,000 people in the United States (1) and 2.5 million people worldwide (2). Over the disease course, a wide variety of disabling symptoms may develop, including motor and sensory disturbance, vision symptoms, cognitive impairment, mood disturbance, pain, and fatigue. These functional deficits and symptoms have a drastic impact on a patient's personal functioning, social interactions, employment, and overall quality of life. Although disease modifying therapies (DMTs) that target primarily the inflammatory immunopathology of MS can slow the development of functional disabilities (3, 4), these do not specifically alleviate symptoms such as cognitive impairment, mood disturbance, pain, and fatigue. Therefore, it is of utmost importance to develop effective and alternative approaches to symptom management.

Recently, transcranial direct current stimulation (tDCS), a form of non-invasive transcranial electrical stimulation, has been probed as a possible form of non-pharmacological intervention in several neurological and psychiatric disorders (5–7), due to its safety, portability, and potential for at-home application. tDCS modulates neuronal transmembrane potential toward hyperpolarization or depolarization by delivering weak electrical currents to the scalp, thereby altering plasticity in the stimulated brain regions (8, 9). These effects have been associated with changes in resting membrane potential, alteration of transmembrane proteins, and N-methyl-d-aspartate receptor efficiency (10, 11). Depending on whether anodal or cathodal stimulation is applied, tDCS either increases or decreases cortical excitability, respectively (12, 13), in turn affecting a wide range of behavioral measures (14, 15). Studies have reported beneficial effects of tDCS on language performance (16), learning processes (17), working memory function (18), and multitasking performance (19) in healthy adults.

Specifically in patients with MS, studies suggest that tDCS could serve as a promising tool to improve cognition (20, 21), neuropathic pain (22, 23), mood (24), and fatigue (25, 26). It has been reported that by applying daily sessions of anodal tDCS for 10 days over the dorsolateral prefrontal cortex (DLPFC) during cognitive training improved attention, information processing and executive function. Further, the improvement was sustained 6 months after last treatment (21). While studies provide intriguing evidence supporting tDCS as a therapeutic strategy for MS patients [reviewed in (27–29)], beneficial effects are not always observed. For example, in a randomized, controlled trial, 1-week tDCS application showed no measurable differences in fatigue score between stimulation and placebo interventions post stimulation (30). A study with three daily tDCS over DLPFC found no effects on mood, fatigue, or attention (22). Another study administering 10 sessions of tDCS also reported that the stimulation and control groups did not differ in standard cognitive measures after the intervention (20).

The methodological discrepancies across these trials have yielded conflicting results and therefore a lack of consensus regarding the effect of tDCS on cognitive impairment, mood disturbance, pain, and fatigue in MS. To enable more definitive conclusions regarding the potential of tDCS as a therapeutic strategy for the described MS-related domains, we performed a systematic review and meta-analysis of the available data.

MATERIALS AND METHODS

Study Identification

Computerized searches were performed in PubMed, Medline, and Web of Science to identify pertinent studies. The search terms were "multiple sclerosis" / "MS" and "transcranial direct current stimulation" / "tDCS." Manual searches of bibliographies of relevant reviews, book chapters, and original articles were also conducted. The searches were limited to human studies published from January 1990 to May 2020 and written in English. Articles were included when the following criteria were met: (1) original research article with a main goal to examine tDCS effects on at least one of the four domains of interest (i.e., cognition, mood, pain, fatigue); (2) the patients were adults with a diagnosis of MS; (3) reports of ≥5 participants receiving tDCS; (4) outcome measures were quantitatively reported; (5) the study included experimental and control conditions. We reviewed the full text of articles that appeared to be relevant.

Quality Assessments

To evaluate the methodological quality of the included studies, we used a modified checklist derived from a quality screening form revised by Moher et al. (31). The quality of each study was evaluated according to the following criteria: (1) random allocation: recorded as 1 if the study pointed out that participants were randomly allocated into different groups; (2) blinding procedure: ranged from 0 to 2, where 0 represented a non-described or non-blinded procedure, and 1 and 2 indicated single-blind and double-blind procedures, respectively; (3) drop-out number: recorded as the number of participants who withdrew from the study; (4) description of baseline demographic data: recorded as 1 when provided; (5) statistical comparison between interventions: denoted as 1 if performed; (6) point estimates and measures of variability: recorded as 1 if provided; (7) adverse effects: recorded as type of the events.

Quantitative Analyses

The relevant information from each study was extracted by one author (W.-Y. H.) using a standard data recording form that included number of participants, MS subtype, mean age, mean/median Expanded Disability Status Scale (EDSS) disease severity score, mean disease duration, stimulation protocol [i.e., duration and intensity of tDCS, targeted brain region(s), method of sham stimulation], domain(s) of measures relevant to current analysis, number of dropouts, study quality (see above), outcome measures, and post-intervention mean (M) as well as standard deviation (SD) for each outcome measure in the experimental and control groups. For studies with multiple measuring points after the intervention, the post-intervention data was based on the first measurement taken after

the intervention period. A wide variety of outcome measures was found across the studies, and some evaluated multiple measures. For the purposes of this meta-analysis, the measure used to assess each study was the explicitly declared primary outcome. If the primary outcome was not clearly defined, the first outcome that was reported in the results section was chosen.

For cognition and mood, one of the studies contributed more than one trial, due to different stimulation sites (24). For fatigue, four articles contributed more than one trial because they applied the stimulation over different brain regions (24, 32, 33) or employed two studies with different design (34). For pain, SD was calculated from standard error of mean (SEM) in one study (23). For fatigue outcome measures, pooled M and SD data were calculated based on subgroup M and SEM in one study (25) and estimated from a subgroup plot in another study (26). One of the studies did not report the M and SD of their outcome measures and the data were extracted from the figures (30). The SD was calculated from SEM (32, 35) and data range (36) based on the range rule of thumb (37, 38) in three of the studies. All the extracted data were carefully checked by another author (C.-H. C.) and disagreements were resolved by discussion.

The analyses were performed with Comprehensive Meta-Analysis 3.0 software (Biostat Inc, Englewood). The standardized effect sizes and 95% confidence interval (CI) were calculated to test the results of different trials. The effect sizes were calculated based on differences between the post-treatment evaluations (22, 24, 25, 32, 33, 36, 39–42), changes relative to the baseline (23), or the mean changes between pre- and post-treatments (20, 21, 26, 30, 34, 35) in the experimental and control groups, divided by the pooled SD. Because the effect sizes from each study may be influenced by the sample sizes, a weighting factor was applied to give more weight to the studies with larger samples. Finally, the mean effect sizes were obtained after combining the weighted effect size of each study. Absolute effect sizes that ranged from 0.2 to 0.49 were considered to be small (43) and a value of 0.5 is likely to be clinically meaningful (44).

The heterogeneity across effect sizes was assessed with Q-statistics (45) and the I^2 index (46), which is useful for assessing consistency between trials (47). When significant heterogeneity was found by Q-statistics or when $I^2 > 50\%$, a random effects model was applied. Otherwise, a fixed effects model was used. Begg and Mazumdar rank correlation (48) was also applied to assess the publication bias. In addition, a funnel plot (49) was used to further address publication bias. In a funnel plot, the effect size is plotted against the standard error. Studies with larger sample sizes appear toward the top of the plot, and near the mean effect size, whereas studies with smaller sample sizes appeared toward the bottom of the plot, indicating more variation in these smaller studies. In the absence of publication bias, the plot may show a symmetrical distribution. Conversely, in the presence of publication bias, the funnel plot would be asymmetrical. The Trim and Fill procedure (50), a funnel plot-derived approach aimed at identifying publication bias and adjusting the results, was applied to correct for publication bias. The significance level was set at $p \leq 0.05$.

RESULTS

Evidence Base

The search yielded 257 records. After duplicates were removed, 135 articles were screened based on title and abstract. Twenty-four potentially relevant articles were obtained for full-text review; 17 articles that met our inclusion criteria were then selected (20–26, 30, 32–36, 39–42). The other seven articles were excluded for the following reasons: review articles or case reports/editorial commentary, applied other types of stimulation, or the main goal of the study was not to assess the effects of tDCS on any of the domains of interest (i.e., cognition, mood, pain, fatigue) (**Figure 1**). **Table 1** summarizes the characteristics of the studies included in our meta-analysis. A total of 383 MS patients were involved, 251 of whom had relapsing-remitting MS. Of the 17 articles, four focused on more than one domain (22, 24, 40, 42). Four studies assessed cognition (20–22, 24). Mood and pain were measured in four (22, 24, 40, 42) and three (22, 23, 42) studies, respectively. Two studies evaluated mood status before and after the intervention, with a purpose to control for mood as a potential confounding factor (23, 30). Fourteen articles evaluated fatigue (22, 24–26, 30, 32–36, 39–42).

Intervention

These studies employed different study designs. Two studies were designed as single session trials (26, 35). Ten studies applied the stimulation at an intensity lower than 2 mA (20, 25, 26, 30, 32, 33, 35, 36, 39, 41). Target stimulation regions included motor cortex (23, 25, 32, 42), dorsolateral prefrontal cortex (20–22, 24, 30, 32, 34, 35, 40), primary somatosensory cortex (33, 36, 39, 41), sensorimotor cortex (33) and parietal cortex (24, 26).

Outcome Measures

A variety of outcome measures was used in the selected articles. For cognition, Attention Network Test (22, 24), Symbol Digit Modalities Test (21) and Brief International Cognitive Assessment for MS (20) were performed. For mood, Hospital Anxiety and Depression Scale (22, 24, 40) and Beck Depression Inventory (42) were included. Pain was assessed with Visual Analog Scale (22, 23, 42). Fatigue was assessed using the Modified Fatigue Impact Scale in eight trials (30, 33, 36, 39–41, 51); other outcome measures for fatigue included Fatigue Impact Scale (25), vigilance task (26), Fatigue Severity Scale (24, 32), Patient-Reported Outcomes Measurement Information System-fatigue short form (34), simple reaction time task (35), and fatigue index (42).

Methodological Quality

Table 2 shows the quality assessment results of the included studies. Random allocation was achieved in all the studies except two trials (20, 34). Most of the studies were of double-blind (21–26, 30, 32–34, 36, 39–42) or single-blind (35) design. Baseline demographic data were described in all the studies. Six studies had drop-outs (20, 24, 25, 33, 34, 41). Statistical comparisons were completed in all the articles; however, one study did not provide point estimates and measures of variability (30). Eight studies reported adverse events. These included skin reaction, insomnia, tingling, itching, phosphene, burning sensation, head

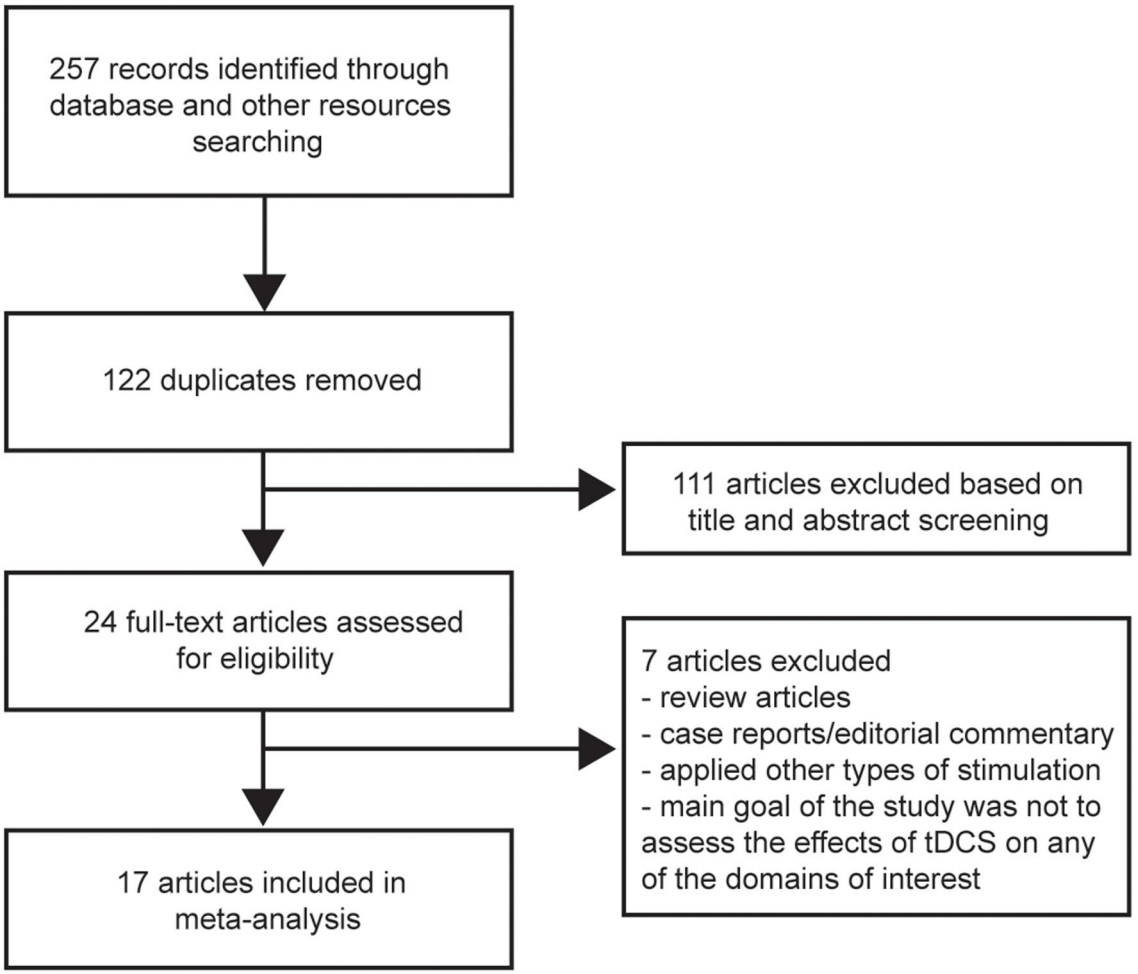

FIGURE 1 | Flow diagram of study identification and inclusion as according to PRISMA guidelines.

pain or pressure, difficulty concentrating, facial muscle twitching, nausea, fatigue, and iron taste (21, 22, 24, 25, 30, 32, 34, 40). One study (23) reported no adverse events.

Meta-Analysis

Table 3 summarizes the domains of measures, outcome measures, the number of participants in the post-treatment evaluations, mean and SD, and effect size of each study.

Cognition

A total of five effect sizes was obtained from four articles with 90 patients (**Table 3**). Since it has been demonstrated that tDCS effects on cognition are task- and cognitive domain-specific (52, 53), we divided the studies into two separate analyses based on the cognitive tasks evaluated: [Symbol Digit Modalities Test (SDMT) vs. Attention Network Test (ANT)], given that SDMT is the most widely used measure of information processing speed in MS (54, 55) and ANT is the most commonly administered task in the five trials. One study that administered the SDMT as part of the Brief International Cognitive Assessment for MS but only reported composite scores (20) was excluded from the subsequent analyses. Therefore, only four trials with a total of 46 patients were included in task-specific analyses. The analyses revealed an effect size of 1.15 (95% CI, 0.20–2.10, $p = 0.01$) for the trial administering the SDMT (21). Mean effect size for trials that applied ANT was −0.49 (95% CI, −0.97 to −0.02, $p = 0.04$) (**Figure 2A**). We did not find heterogeneity among the studies that applied ANT ($Q = 3.42$, $I^2 = 41.55$, $p = 0.18$). Heterogeneity analysis was not applicable for SDMT since only one trial was included. Publication bias was not found based on rank correlation (tau = −0.30, $p = 0.46$) when considering all five trials investigating tDCS effects on cognition. The funnel plot resembles an inverted symmetrical funnel, which confirmed that publication bias is absent (**Figure 3A**).

Mood

Four effect sizes were obtained from three articles with a total of 32 patients for mood. A strong mean effect size of 0.92 (95% CI, −0.03–1.88, $p = 0.05$) (**Figure 2B**) was found. There was heterogeneity across the studies ($Q = 12.08$, $I^2 = 75.17$, $p = 0.007$). The results of rank correlation (tau = 0.33, $p = 0.49$) and the symmetrical funnel plot (**Figure 3B**) indicate that publication

TABLE 1 | Characteristics of each study included in the meta-analysis.

Study	Number of participants (stim/sham)	MS subtype	Mean age (years) (stim/sham)	Mean/median EDSS (stim/sham)	DD (years) (stim/sham)	Stimulation form and protocol	Stimulation position and electrode size	Method of sham stimulation	Domain of measures relevant to current analysis
Charvet et al. (20)¶	45 (25/20)[a]	22 RR	52.6/51.0	N/R	17.7/15.7	atDCS 1.5 mA 20 min daily for 10 days	A: L DLPFC (35 cm^2) Ref: R DLPFC (35 cm^2)	N/A	Cognition
Mattioli et al. (21)¶	20 (10/10)	20 RR	38.2/47.4	2.1/2.9	6.6/11	atDCS 2 mA 20 min daily for 10 days	A: L DLPFC (25 cm^2) Ref: R shoulder (60 cm^2)	30 s of stimulation at the beginning and the end of the session	Cognition
Ayache et al. (22)*	16 (16/16)	11 RR 4 SP 1 PP	48.9/48.9	4.25/4.25	11.8/11.8	atDCS 2 mA 20 min daily for 3 days	A: L DLPFC (25 cm^2) Ref: R supraorbital (25 cm^2)	Ramped down immediately after ramping up	Pain mood cognition fatigue
Mori et al. (23)	19 (10/9)	19 RR	42.8/46.3[§]	1.5/2[§]	10.1/10.3[§]	atDCS 2 mA 20 min daily for 5 days	A: primary motor cortex contralateral to the somatic painful area (35 cm^2) Ref: contralateral supraorbital region (35 cm^2)	Stimulator was turned off after 30 s of stimulation	Pain
Chalah et al. (24)*	10 (10/10)[b]	9 RR 1 SP	40.5/40.5	2.3/2.3	14/14	atDCS 2 mA 20 min daily for 5 days	(1) A: L DLPFC (25 cm^2) Ref: R supraorbital region (25 cm^2) (2) A: R PPC (25 cm^2) Ref: Cz (25 cm^2)	Ramped up for 15 s followed by 30 s of stimulation and a ramping down period of 15 s	Fatigue cognition mood
Ferrucci et al. (25)*	25 (25/25)	22 RR 3 SP	44.5/44.5[†]	3.2/3.2[†]	13.2/13.2[†]	atDCS 1.5 mA 15 min daily for 5 days	A: bilateral motor cortex (35 cm^2) Ref: R deltoid (35 cm^2)	Stimulator was turned off after 10 s of stimulation	Fatigue
Hanken et al. (26)	46 (23/23)	18 RR 28 SP	51.3/46.8[c]	4.4/3.95[c]	11.5/12.7[c]	atDCS 1.5 mA for 20 min	A: R parietal cortex (35 cm^2) Ref: contralateral forehead (35 cm^2)	Ramped up for 8 s followed by 30 s of stimulation and a ramping down period of 5 s, and then every 550 ms, a current of 110 μA was released	Fatigue
Saiote et al. (30)*	13 (13/13)	13 RR	46.8/46.8	3.5/3.5	9/9	atDCS 1 mA 20 min daily for 5 days	A: L DLPFC (35 cm^2) Ref: contralateral forehead (90 cm^2)	Ramped down immediately after ramping up	Fatigue
Mortezanejad et al. (32)	36 (12/12)[d] (12/12)[e]	N/R	33.3/32.5[d] 32.0/32.5[e]	1.75/1.37[d] 1.46/1.37[e]	N/R	atDCS 1.5 mA 20 min daily for 6 days	(1) A: L M1 (35 cm^2) Ref: contralateral supraorbital region (35 cm^2) (2) A: L DLPFC (35 cm^2) Ref: contralateral supraorbital region (35 cm^2)	Stimulator was turned off after 30 s of stimulation	Fatigue

(Continued)

TABLE 1 | Continued

Study	Number of participants (stim/sham)	MS subtype	Mean age (years) (stim/sham)	Mean/median EDSS (stim/sham)	DD (years) (stim/sham)	Stimulation form and protocol	Stimulation position and electrode size	Method of sham stimulation	Domain of measures relevant to current analysis
Tecchio et al. (33)*	13 (13/13)[f] 8 (8/8)[g]	21 RR	45.8/45.8[f] 38.1/38.1[g]	1.5/1.5[f] 2/2[g]	7.6/7.6[f] 13.5/13.5[g]	atDCS 1.5 mA 15 min daily for 5 days	(1) A: bilateral SI_{wb} (35 cm^2) Ref: Oz (84 cm^2) (2) A: bilateral $SM1_{hand}$ 35 cm^2 Ref: under the chin (84 cm^2)	4 s of stimulation at the beginning and the end of the session	Fatigue
Charvet et al. (34)	35 (15/20)[h] 27 (15/12)[i]	18 RR[h] 13 RR[i]	53.4/51.0[h] 44.8/43.4[i]	6/4[h] 6/3.5[i]	15.6/15.7[h] 15.8/13.3[i]	[h]atDCS 1.5 mA 20 min daily for 10 days [i]atDCS 2 mA 20 min daily for 20 days	A: L DLPFC (25 cm^2)	[i]Ramp up to 2.0 mA and back down during the first and last minutes of the session	Fatigue
Fiene et al. (35)*	15 (15/15)	14 RR 1 SP	43.2/43.2	3.54/3.54	9.63/9.63	atDCS 1.5 mA for a mean duration of 27.29 min	A: L DLPFC (25 cm^2) Ref: R shoulder (35 cm^2)	Current turned off after 30 s with a ramp-down of 15 s	Fatigue
Porcaro et al. (36)*	18 (18/18)	18 RR	44.5/44.5	1.1/1.1	6.9/6.9	atDCS 1.5 mA 15 min daily for 5 days	A: bilateral SI_{wb} (35 cm^2) Ref: Oz (70 cm^2)	4 s of stimulation at the beginning and the end of the session	Fatigue
Cancelli et al. (39)*	10 (10/10)	10 RR	43.2/43.2	0.9/0.9	6.6/6.6	atDCS 1.5 mA 15 min daily for 5 days	A: bilateral SI (35 cm^2) Ref: Oz (70 cm^2)	4 s of stimulation at the beginning and the end of the session	Fatigue
Chalah et al. (40)*	11 (11/11)	10 RR 1 SP	43.9/43.9	3.14/3.14	6.3/6.3	atDCS 2 mA 20 min daily for 5 days	A: L DLPFC (35 cm^2) Ref: R DLPFC (35 cm^2)	Ramped up for 15 s followed by 30 s of stimulation and a ramping down period of 15 s	Fatigue mood
Tecchio et al. (41)*	10 (10/10)	7 RR 1 SP 2 PP	45.8/45.8	1.5/1.5	7.1/7.1	atDCS 1.5 mA 15 min daily for 5 days	A: bilateral SI (35 cm^2) Ref: Oz (70 cm^2)	4 s of stimulation at the beginning and the end of the session	Fatigue
Workman et al. (42)*	6 (6/6)	6 RR	46.7/46.7	N/R	N/R	atDCS 2 mA 20 min daily for 5 days	A: M1 representation of the more-affected leg (35 cm^2) Ref: contralateral supraorbital region (35 cm^2)	Ramped up to 2 mA and then the current was set to 0 mA	Pain fatigue mood

stim, stimulation group; sham, sham group; EDSS, Expanded Disability Status Scale; DD, disease duration; RR, relapsing-remitting; SP, secondary-progressive; PP, primary-progressive; atDCS, anodal transcranial direct current stimulation; A, anode; Ref; reference; SI, primary somatosensory cortex; SI_{wb}, whole body somatosensory areas; $SM1_{hand}$, hand sensorimotor areas; PPC, posterior parietal cortex; M1, primary motor cortex; DLPFC, dorsolateral prefrontal cortex; R, right; L, left; N/R, not reported; N/A not applicable.

*¶tDCS was paired with cognitive training. *Cross-over design. [§]Data calculated from Mori et al. (23), Table 1. [†]Data calculated based on 23 participants included in the final analysis in Ferrucci et al. (25), Table 1. [a]Participants in the control group did not receive either tDCS or sham stimulation. [b]Data from 10 participants included in the final analysis in Chalah et al. (24). [c]Data calculated based on 40 participants included in the final analysis in Hanken et al. (26), Table 4. [d]Participants in M1 group. [e]Participants in L DLPFC group. [f]Participants in SI_{wb} group. [g]Participants in $SM1_{hand}$ group. [h]Study 1, open-label study. Twenty participants only participated in cognitive training and did not receive either tDCS or sham stimulation. [i]Study 2, randomized controlled trial.*

TABLE 2 | Quality assessment for studies included in the meta-analysis.

Study	Random allocation	Blinding	Baseline demographic data	Drop-outs	Between conditions statistical comparison	Point estimates and variability	Adverse effects
Charvet et al. (20)	0	0	1	1	1	1	N/R
Mattioli et al. (21)	1	2	1	0	1	1	Itchiness, pain, burning, warmth, pinching, fatigue, iron taste
Ayache et al. (22)	1	2	1	0	1	1	Insomnia, nausea, headache, phosphene
Mori et al. (23)	1	2	1	0	1	1	None
Chalah et al. (24)	1	2	1	2	1	1	Insomnia, headache
Ferrucci et al. (25)	1	2	1	2	1	1	Skin reaction
Hanken et al. (26)	1	2	1	0	1	1	N/R
Saiote et al. (30)	1	2	1	0	1	0	Headache, skin sensation
Mortezanejad et al. (32)	1	2	1	0	1	1	Tingling, itching
Tecchio et al. (33)	1	2	1	2	1	1	N/R
Charvet et al. (34)	0	0	1	0	1	1	Tingling, itching, burning sensation, head pain or pressure, difficulty concentrating, facial muscle twitching, nausea
	1	2	1	2	1	1	
Fiene et al. (35)	1	1	1	0	1	1	N/R
Porcaro et al. (36)	1	2	1	0	1	1	N/R
Cancelli et al. (39)	1	2	1	0	1	1	N/R
Chalah et al. (40)	1	2	1	0	1	1	Phosphene, sleep disturbance
Tecchio et al. (41)	1	2	1	1	1	1	N/R
Workman et al. (42)	1	2	1	0	1	1	N/R

N/R, not reported.

bias did not seem to affect the validity of the overall effect size obtained by the meta-analysis of mood. Two studies evaluating mood as a control, rather than outcome variable, were not included in the meta-analysis (23, 30). Mood status was measured by Chalah et al. (40) but the effect sizes could not be determined since point estimates for the control group were not reported.

Pain

Three effect sizes were determined for pain from three articles with a total of 41 patients. A moderate mean effect size of 0.59 (95% CI, 0.08–1.10, $p = 0.02$) (**Figure 2C**) was discovered. We did not find heterogeneity among the studies ($Q = 3.49$, $I^2 = 42.82$, $p = 0.17$). Publication bias was not found by either rank correlation (tau = 0.00, $p = 1.00$) or the funnel plot (**Figure 3C**).

Fatigue

A total of 18 effect sizes were extracted from 14 articles (with 291 patients), and the mean effect size was 0.60 (95% CI, 0.31–0.89, $p < 0.001$) (**Figure 2D**). Heterogeneity was observed across studies ($Q = 38.45$, $I^2 = 55.79$, $p = 0.002$). Publication bias was discovered by rank correlation (tau = 0.39, $p = 0.02$) and an asymmetrical funnel plot showing a higher concentration of studies on one side of the mean than the other (**Figure 3D**). Therefore, a planned Trim and Fill procedure (50) was applied to impute missing studies. After adjusting for missing studies, a mean effect size of 0.39 was found.

Since a larger number of effect sizes (i.e., 18) was extracted for fatigue, we explored whether other variables would influence the measured effect. To achieve this, we performed subgroup analyses based on stimulation intensity (low: <2 mA vs. high: ≥2 mA) and outcome measures [Fatigue Severity Scale (FSS) vs. Modified Fatigue Impact Scale (MFIS) vs. other outcomes for fatigue] that were applied in the studies. The subgroup analysis of stimulation intensity revealed a mean effect size of 0.62 (95% CI, 0.05–1.19, $p = 0.03$) for six trials from five studies (22, 24, 34, 40, 42) with a "high" intensity (i.e., ≥2 mA). Mean effect size for 12 trials from 10 studies (25, 26, 30, 32–36, 39, 41) with "low" intensity (i.e., <2 mA) was 0.60 (95% CI, 0.25–0.95, $p = 0.001$). For the analysis of outcome measures, a mean effect size of 1.14 (95% CI, 0.68–1.60, $p < 0.001$) was found for FSS [four trials (24, 32)]. The mean effect sizes for MFIS [eight trials (22, 30, 33, 36, 39–41)] and other fatigue outcomes [six trials, including Fatigue Impact Scale (25), vigilance task (26), Patient-Reported Outcomes Measurement Information System-fatigue short form (34), simple reaction time task (35), and fatigue index (42)] were 0.31 (95% CI,

TABLE 3 | Summary of the effect sizes.

Domain of measures	Study	Outcome measures	Nexp/Nctrl	Mexp/Mctrl	SDexp/SDctrl	ES
Cognition	Charvet et al. (20)	BICAMS	24/20	0.09/0.09	0.47/0.47	0.00
	Mattioli et al. (21)	SDMT	10/10	8.8/−0.1	8.6/6.7	1.15
	Ayache et al. (22)	ANT (alertness)	16/16	52.1/58.8	36/66	−0.12
	Chalah et al. (24)	ANT[a] (mean reaction time)	10/10	660.2/620.6	29.7/34	−1.24
		ANT[b] (mean reaction time)	10/10	634.7/620.6	26.2/34	−0.46
Mood	Ayache et al. (22)	$HADS_{total}$	16/16	13.6/14.5	5.8/6.5	0.14
	Chalah et al. (24)	$HADS_{anxiety}$a	10/10	2.8/3.8	0.5/1.0	1.26
		$HADS_{anxiety}$b	10/10	2.0/3.8	0.5/1.0	2.27
	Workman et al. (42)	BDI	6/6	11.5/9.8	12.1/7.0	0.17
Pain	Ayache et al. (22)	VAS	16/16	43.1/50.3	26.2/19.7	0.31
	Mori et al. (23)	VAS	10/9	45.5/89.3	34.7/25.8[c]	1.42
	Workman et al. (42)	VAS	6/6	11.3/18.8	12.8/34.5	0.28
Fatigue	Ayache et al. (22)	MFIS	16/16	49.0/47.4	15.2/17.7	−0.09
	Chalah et al. (24)	FSS[a]	10/10	3.3/3.9	0.4/0.5	1.32
		FSS[b]	10/10	3.8/3.9	0.5/0.5	0.20
	Ferrucci et al. (25)	FIS	23/23	46.3/46.3[d]	21.6/26.9[d]	0.00
	Hanken et al. (26)	Vigilance task	20/20	−20/35[e]	84.71/71.46[e]	0.70
	Saiote et al. (30)	MFIS	13/13	0.5/−3[f]	5.4/4.5[f]	−0.7
	Mortezanejad et al. (32)	FSS[g]	12/12	3.79/4.71	0.51/0.51[c]	1.80
		FSS[h]	12/12	3.55/4.71	1.07/0.51[c]	1.38
	Tecchio et al. (33)	MFIS[i]	13/13	31.0/34.7	12.0/10.4	0.33
		MFIS[j]	8/8	42.1/52.1	17.2/22.0	0.50
	Charvet et al. (34)	PROMIS-Fatigue short form[k]	15/20	−2.5/−0.2	7.4/5.3	0.36
		PROMIS-Fatigue short form[l]	15/12	−5.6/0.9	8.9/1.9	0.95
	Fiene et al. (35)	Simple reaction time task	15/15	−2.76/6.99	14.0/18.5[c]	0.59
	Porcaro et al. (36)	MFIS	18/18	32.5/41.4	11.5/10.7[m]	0.80
	Cancelli et al. (39)	MFIS	10/10	27.6/46.0	19.4/18.6	0.96
	Chalah et al. (40)	MFIS	11/11	39.27/41.73	22.0/19.3	0.11
	Tecchio et al. (41)	MFIS	10/9	31.0/34.8	4.0/3.5	1.00
	Workman et al. (42)	Fatigue index	6/6	50.1/72.3	11.9/11.3	1.91

N, number of patients in the post-treatment evaluation; exp, experimental group; ctrl, control group; M, mean; SD, standard deviation; ES, effect size; ANT, Attention Network Test; SDMT, Symbol Digit Modalities Test; BICAMS, Brief International Cognitive Assessment for MS; HADS, Hospital Anxiety and Depression Scale; BDI, Beck Depression Inventory; VAS, Visual Analog Scale; FIS, Fatigue Impact Scale; PROMIS, Patient-Reported Outcomes Measurement Information System; MFIS, Modified Fatigue Impact Scale; FSS, Fatigue Severity Scale. [a]*Left dorsolateral prefrontal group.* [b]*Right posterior parietal cortex group.* [c]*Data calculated from standard error of the mean.* [d]*Pooled data were calculated based on subgroup mean and standard error of mean listed in Table 2, Ferrucci et al. (25).* [e]*Data from Figure 5, Hanken et al. (26).* [f]*Data from Figure 3, Saiote et al. (30).* [g]*Motor cortex group.* [h]*Dorsolateral prefrontal cortex group.* [i]*Data for SI_{wb} group.* [j]*Data for $SM1_{hand}$ group.* [k]*Data from open-label study.* [l]*Data from randomized controlled trial.* [m]*Data calculated from data range based on range rule of thumb.*

0.03–0.60, $p = 0.03$) and 0.53 (95% CI, 0.23–0.82, $p < 0.001$), respectively.

DISCUSSION

The results of this meta-analysis suggest that tDCS might be helpful in improving cognition (processing speed), mood disturbance, pain, and fatigue in MS. There has been increasing interest in treatment strategies to improve cognitive impairment (56). Here, we found a strong effect size of 1.15 for the trial that administered SDMT, and a negative effect for the trials that used ANT (effect size = −0.49). The results suggest that tDCS-induced cognitive improvement is task-specific or cognitive domain-specific. However, the findings should be interpreted with caution given the small sample size. SDMT is a widely used test in MS clinical trials and mainly evaluates information processing speed and immediate visual memory recall. Since cognitive processing speed is the most commonly affected cognitive domain (57, 58), it is possible that the test is more sensitive to detect cognitive improvements, including changes induced by tDCS. It is unclear why the performance of ANT was not improved by tDCS. One possibility is that the stimulation duration might not have been optimal. For instance, in the trial that administered SDMT and showed positive effects, 10 sessions of stimulation were applied (21). However, in studies using ANT as an outcome, no more than five sessions of stimulation were employed (22, 24). Study design may also affect the results: the study administering SDMT delivered tDCS during cognitive training, whereas the studies using ANT did not pair the stimulation with cognitive tasks. Another possible explanation is that baseline cognitive

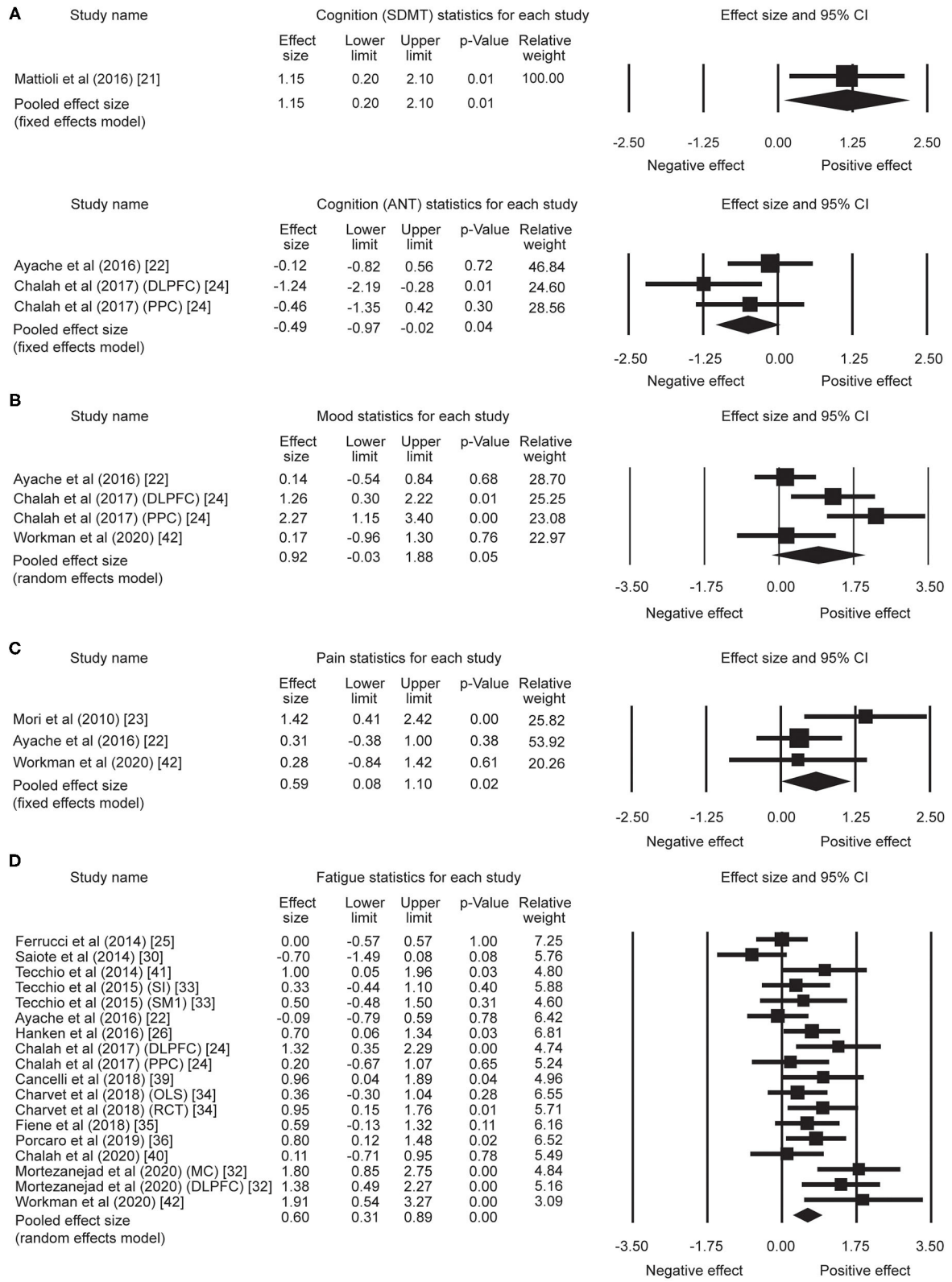

FIGURE 2 | Statistical summary and forest plot of effect sizes for **(A)** cognition, **(B)** mood, **(C)** pain, and **(D)** fatigue outcome measures. SDMT, Symbol Digit Modalities Test; ANT, Attention Network Test; CI, confidence interval; DLPFC, dorsolateral prefrontal cortex; PPC, posterior parietal cortex; SI, whole body somatosensory areas; SM1, hand sensorimotor areas; OLS, open-label study; RCT, randomized controlled trial; MC, motor cortex.

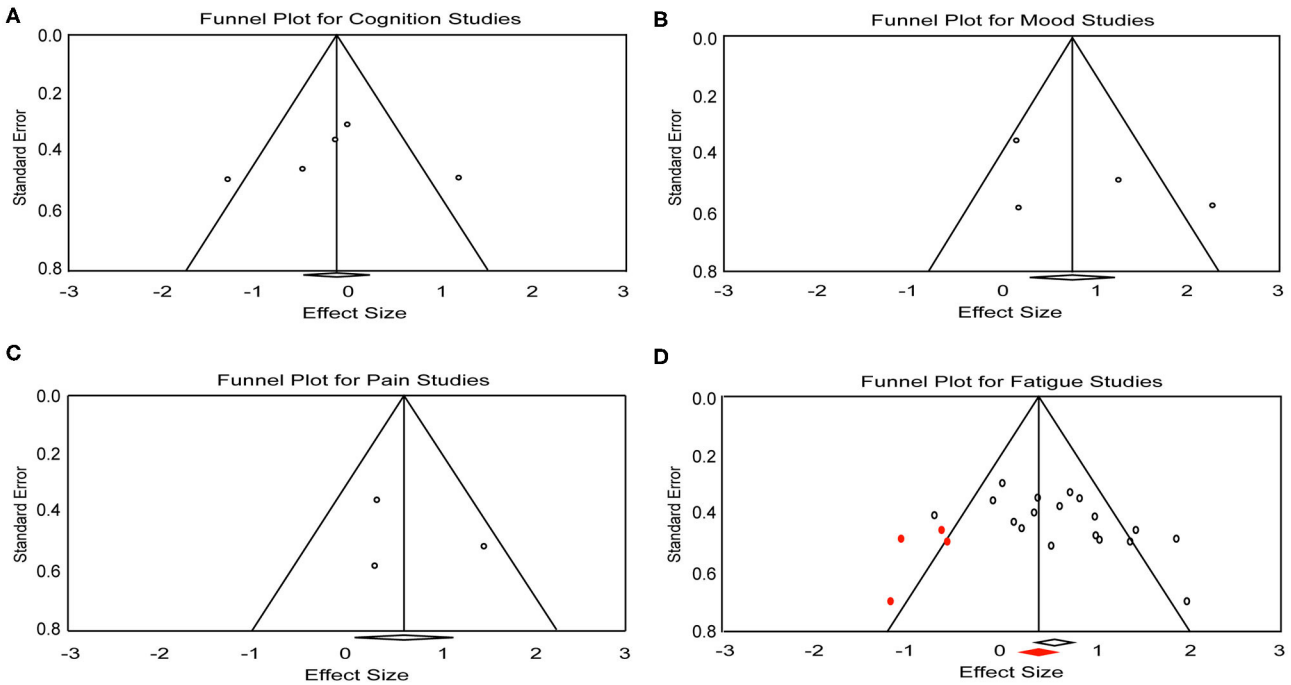

FIGURE 3 | Funnel plot for **(A)** cognition, **(B)** mood, **(C)** pain, and **(D)** fatigue studies included in the meta-analysis. Red dots represent the imputed missing studies. Red rhombus shows the adjusted mean effect size.

performance is a critical factor in determining whether tDCS—or any cognitive intervention—enhances cognitive performance (59, 60). Since most of the studies included in this meta-analysis did not specifically recruit patients with cognitive impairment, the heterogeneity in cognitive performance across participants may have affected the results. Further investigation with more homogeneous patient populations, different stimulation protocols, and cognitive assessments is needed to draw a conclusion regarding the optimal stimulation protocol and the effect of tDCS on different dimensions of cognition.

A strong mean effect size of 0.92 was discovered for mood disturbance. Further, studies that measured pain showed a mean effect size of 0.59, which is clinically meaningful (44). Neuropathic pain is one of the most common symptoms (61) and it is thought to be a consequence of maladaptive plastic changes within the nociceptive system which alters nociceptive signal processing (62). Studies have suggested that pain decreased by tDCS may be the result of functional changes in brain structures that are critical in pathogenesis of neuropathic pain (22, 23). By acting on pain-related corticosubcortical and corticocortical pathways, tDCS modulates perception of pain and reduces chronic neuropathic pain. However, further studies are warranted to better differentiate tDCS effects on neuropathic and nociceptive pain. While the results suggested beneficial effects of tDCS on mood disturbance and pain, the findings should be viewed conservatively since the sample size is small (mood: 32 patients; pain: 41 patients).

The mean effect size for fatigue was 0.60. A subgroup analysis was conducted to explore whether stimulation intensity and outcome measures being applied would influence the measured effect for fatigue. Both high and low intensities of stimulation demonstrated moderate effect sizes (high: effect size = 0.62; low: effect size = 0.60), suggesting that high and low intensities could yield nearly the same level of favorable effects on fatigue. Interestingly, graded stimulation effects were reported previously, where a larger learning effect was observed in healthy adults when the stimulation is applied at a higher intensity (63). Given that chronic inflammatory activity (64) and central inflammation (65) are related to synaptic plasticity, it is possible that how the brain responds to the tDCS intervention is altered. In this scenario, stimulation could lead to qualitatively different outcomes in intact vs. dysfunctional neural circuits. In contrast to the findings in healthy adults, we found that both high and low stimulation intensities relieved fatigue, with a similar degree of effect. Subgroup analysis of outcome measures demonstrated a relatively higher effect size for trials using the FSS (effect size = 1.14) than those using the MFIS (effect size = 0.31) and other outcomes assessments (effect size = 0.53), indicating that the FSS may be more sensitive to detect changes in fatigue induced by tDCS. Both the FSS and MFIS are widely used in assessing fatigue, but the item contents of the two scales are different. While the FSS primarily targets physical aspects of fatigue, MFIS measures physical, cognitive and psychosocial fatigue. Since the two scales measure different aspects of fatigue (66), the observed larger effect size for trials using the FSS suggests that tDCS effects may be more beneficial to treat physical fatigue. Physical fatigue in MS is associated with a progressive disease course and greater physical disability (67). Often, the impact of physical dysfunction

on daily activities can be recognized more easily than that of mental fatigue. However, it is unclear how reliably a patient can actually distinguish between physical and mental fatigue, since perceived mental or physical fatigue does not correlate with objective measures of cognitive or physical performance (68, 69). Thus, further studies in a larger population are required to better determine the most sensitive outcome measures for detecting tDCS effects on fatigue.

One important consideration for this systematic review and meta-analysis is the methodological quality of the selected studies. Most of the trials included did achieve random allocation, and reported control groups and blinding procedures. However, two studies measuring tDCS effects on fatigue provided no point estimates or measures of variability, and these data were estimated from their figures (26, 30). The influence of non-precise data on the mean effect size cannot be fully excluded. Further, possible publication bias was detected in studies for fatigue. Although a Trim and Fill procedure (50) was performed to adjust the mean effect size, the results obtained in the present meta-analysis must be viewed conservatively. Despite the funnel plot and rank correlation analyses both indicating there was no publication bias in the studies for cognition, mood and pain, bias could not be fully excluded since the small number of trials included could limit the bias detection.

While tDCS is generally thought to be safe for both healthy adults and clinical populations, and no severe adverse effects have been reported, investigators should adhere to safety guidelines (70) and conduct follow-up assessments to monitor longer-term risks and benefits. In addition to safety concerns, several crucial questions should be addressed in future studies with proper experimental design. First, it is essential to elucidate the underlying neural mechanisms of positive effects on cognition, mood, pain, and fatigue induced by the tDCS. Second, further investigation is needed for optimizing stimulation protocols and finding the most effective parameters to apply tDCS as a treatment approach for MS. Third, studies with subgroups that are varied in subtypes of MS and clinical severity are necessary to identify the subgroups of patients most likely to benefit from tDCS. Studies have demonstrated that the efficacy of non-invasive electrical stimulation is correlated with the magnitude of the electric field that reaches the targeted brain area, which highlights the importance of anatomical variability and individualizing stimulation protocols (71–73). Thus, inter-individual variability in response to tDCS should be taken into account.

Some limitations exist in the review. First, it is difficult to estimate potential confounders such as regimens and types of DMTs, disease evolution profiles and effects of medicinal products. In the studies included in the meta-analysis, mood, pain, and fatigue were mainly measured with patient-reported outcome measures, which have very little or no motor component involved. For cognition, a motor component was involved in performing the task. However, how motor function, and other factors such as spasticity and fatigue, could have influenced the cognitive performance was not explicitly discussed. Second, we may have missed relevant studies that were published in non-English languages. Third, the findings of the current study should be taken with caution given the relatively small sample size and the repeated analyses in the same domain (e.g., ANT task) with the same patient population. The fact that relapsing-remitting MS was the majority population also makes it difficult to provide information about differences in treatment response between MS subtypes. Finally, methodological variations existed between the selected studies with respect to outcome measures, patient inclusion criteria, experimental design (e.g., cross-over vs. parallel design), and tDCS protocols. For instance, in studies measuring fatigue, the number of stimulation sessions varied across trials, with a range from single session to 20 sessions. Previous studies have reported that repeated sessions of tDCS can result in cumulative effects (74, 75). Although trials applied 20 sessions of tDCS (34) did not show a larger ES (0.95) compared to trials with five or six sessions of stimulation (ES ranging from −0.7 to 1.91), the influence of heterogeneity across the studies on the effect estimation cannot be ruled out. Stimulation timing ("online" vs. "offline") and intervals between stimulation sessions are also critical factors that may affect the observed effects. However, subgroup analyses based on these factors are not suitable given the low number of total studies included, which limited us to simply determine the different degrees of the effect generated by timing of the stimulation and stimulation intervals.

In conclusion, this meta-analysis suggests preliminary evidence of favorable effects of tDCS on cognition, mood disturbance, pain, and fatigue in MS. For cognition, tasks targeting cognitive aspects including processing speed, may be more suitable to reflect tDCS-enhanced cognitive performance. For fatigue, applying high and low intensities of stimulation generate nearly the same grade of beneficial effects, and a relatively higher effect size was noted in studies using FSS as an outcome, suggesting that it may be more sensitive in capturing tDCS-induced changes in fatigue. Further well-designed studies are necessary to determine the neural plasticity changes induced by tDCS, optimize stimulation protocol and identify the subgroups of patients who would benefit most.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

W-YH: conceptualization, methodology, data analysis, visualization, and manuscript writing. C-HC: data curation and content curation. TZ, AG, and RB: conceptualization and manuscript editing. All authors: contributed to the article and approved the submitted version.

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BRIEF RESEARCH REPORT
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The Brain-Derived Neurotrophic Factor Val66Met Polymorphism Can Protect Against Cognitive Impairment in Multiple Sclerosis

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Introduction: Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family, involved in neuronal survival and synaptic plasticity. The BDNF Val66Met polymorphism is known to reduce BDNF expression and secretion; its role in multiple sclerosis (MS) is poorly investigated.

Objectives and Methods: In this multicenter, retrospective study, we assessed the role of BDNF Val66Met polymorphism on cognitive and motor disability in MS patients consecutively referred to the University of Florence and the Hospital of Barletta. All patients underwent a genetic analysis for the presence of Val66Met polymorphism and a comprehensive neuropsychological examination on the Rao's Brief Repeatable Battery and the Stroop Color Word Test. Possible predictors of the Expanded Disability Status Scale (EDSS) score and number of failed neuropsychological tests were assessed through linear multivariable regression models.

Results: Ninety-eight patients were recruited. Patients with the BDNF Val66Met polymorphism (35.7%) were more frequently males ($p = 0.020$), more disabled ($p = 0.026$) and, marginally, older ($p = 0.064$). In the multivariable analysis, BDNF Val66Met polymorphism was associated with a better cognitive performance ($B = -1.1 \pm 0.5$, $p = 0.027$). Higher EDSS score was associated with a progressive disease course ($B = 3.4$, $p < 0.001$) and, marginally, with the presence of the BDNF Val66Met polymorphism ($B = 0.56$, $p = 0.066$).

Discussion: Our results preliminarily suggest a protective role of BDNF Val66Met polymorphism against cognitive impairment in MS patients, possibly related to a detrimental effect of increased BDNF concentration in a neuroinflammatory environment.

Keywords: multiple sclerosis, cognitive impairment, disability, brain derived neurotrophic factor, polymorphism

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disorder of the central nervous system (CNS) that affects mainly patients between 20 and 40 years of age. It is the second cause of neurological disability in the young adult population, after trauma (1). Cognitive impairment (CI) is widely acknowledged as a core feature of MS, affecting up to 70% of the patients, with a significant functional impact in everyday activities (2). In adult patients, information processing speed, attention, working and episodic memory, executive functions, and visuospatial abilities are the cognitive domains most commonly impaired, with relative sparing of language and general intelligence (2, 3). CI in MS has been linked to different risk factors (4): among genetic factors, the role of brain-derived neurotrophic factor (BDNF) polymorphisms is receiving growing attention.

BDNF is a member of the neurotrophin family, which also includes nerve growth factor and neurotrophins 3 and 4. BDNF is secreted from dendrites to axons and from axons to dendrites, in autocrine loops, and across long distances through neural circuits (5, 6). BDNF is involved in different processes within the brain, such as plasticity, neuronal survival, formation of new synapses, dendritic branching, and modulation of excitatory and inhibitory neurotransmitter profiles (7).

The BDNF single-nucleotide polymorphism *rs6265* (also named Val66Met) determines the substitution of valine with methionine at codon 66 of the BDNF pro-protein (8). Its presence leads to interference with BDNF intracellular trafficking and secretion, as it has been demonstrated in *in vitro* studies (8, 9). The presence of the abovementioned polymorphism also results in an 18–30% reduction in BDNF secretion (9). The Val66Met polymorphism has been reported to be a risk factor for neurodegenerative disorders (such as Alzheimer's disease) in the adult age (10). In addition, it has been associated with CI in otherwise healthy individuals, particularly with involvement of episodic and working memory, which require neuroplasticity, and hence abundant expression of BDNF in related brain areas (8, 11–14).

In neuroinflammation, the role of BDNF is entangled with the effects of factors involved in the innate and adaptive immune response in neurodegenerative and autoimmune disorders (15), inducing BDNF expression and secretion by immune cells. The role of BDNF in neuroinflammatory disorders, and especially in MS, has been poorly investigated so far, with conflicting results (16). In the first study assessing the role of BDNF Val66Met polymorphism on magnetic resonance imaging (MRI) parameters in a group of MS patients, Met carriers showed a higher risk of developing gray matter (GM) atrophy (17). Conversely, other subsequent studies showed that Met carriers had a higher preservation of brain volume (18) and global and regional GM volumes (19, 20). On the other hand, a large-scale Norwegian study found no role of the BDNF Val66Met polymorphism on clinical and neuropsychological variables (21).

With this background, the aim of the present cross-sectional multicenter study was to assess the influence of Val66Met polymorphism on both cognitive and motor disability in a sample of MS patients.

MATERIALS AND METHODS

Subjects

Patients with MS consecutively referred to the MS Centres at the University of Florence and the Hospital of Barletta between 2014 and 2019 were screened for inclusion. Inclusion criteria were as follows: diagnosis of MS according to the 2010 McDonald's Diagnostic Criteria (22), relapsing-remitting (RR), or progressive (either primary progressive, PP, or secondary progressive, SP) disease course; age >18 years; and no history of intellectual disability, psychosis, or dementia. Exclusion criteria were corticosteroid treatment in the 30 days before inclusion and inability or refusal to perform the blood sampling required for the study purposes. The study was approved by the local Ethic Committees, and written informed consent was obtained by all the subjects.

Clinical and Neuropsychological Examination

In each center, demographic and clinical data were prospectively collected every 6 months and in occasion of relapses and stored in an electronic database (23). For this cross-sectional analysis, at the time of assessment and blood sampling, the following demographic and clinical data were collected by a qualified neurologist: age, sex, education, age at disease onset, disease course, ongoing treatments, relapses in the last year, and disability level as measured on the Expanded Disability Status Scale (EDSS) (24). A well-trained psychologist administered the Brief Repeatable Battery of Neuropsychological Tests (BRB) (25) and the Stroop Color Word Test (SCWT). The BRB assesses the cognitive domains most frequently impaired in MS and incorporates tests of verbal memory [Selective Reminding Test (SRT)], visuo-spatial memory [10/36 Spatial Recall Test (SPART)], complex attention and information processing speed [Paced Auditory Serial Addition Test (PASAT) and Symbol Digit Modalities Test (SDMT)], and verbal fluency [Word List Generation (WLG)]. The SCWT (26) assesses complex attention and aspects of executive functioning such as the ability to inhibit cognitive interference. Failure of a test was defined as a score below the 5th or above the 95th percentile (1.65 SD), as appropriate, on the basis of Italian normative values after adjustment for age, sex, and education (27). Premorbid intelligent quotient (IQ) was estimated through the Italian version of the National Adult Reading Test (NART)—the "Test di Intelligenza Breve" (28). Finally, fatigue and depression were assessed through the Fatigue Severity Scale (29) and the Montgomery and Asberg Depression Rating Scale (30), respectively.

Genetic Analysis

A blood sample for genetic analysis of the BDNF Val66Met polymorphism was obtained from each patient. The presence of the *rs6265* polymorphism was analyzed by first extracting the DNA from peripheral blood samples, using a standardized,

TABLE 1 | Characteristics of the study sample.

	Whole sample ($n = 98$)	BDNF Val/Met ($n = 35$)	BDNF Val/Val ($n = 63$)	p
Females, *n* (%)[a]	65 (66.3%)	18 (51.4%)	47 (74.6%)	**0.020**
Age, mean years (SD)[b]	43.9 (10.9)	46.7 (10.7)	42.3 (10.8)	0.064
Education, mean years (SD)[b]	12.1 (3.96)	11.5 (3.8)	12.5 (4.0)	0.213
IQ, median (IQR)[c]	108.8 (101.6–111.9)	108.2 (98.0–114.0)	109.3 (103.6–111.4)	0.885
Disease course				0.392
RR, *n* (%)[a]	80 (81.6)	27 (77.1)	53 (84.1)	
CP, *n* (%)[a]	18 (18.4)	8 (22.9)	10 (15.9)	
Age at onset, mean years (SD)[b]	35.3 (10.4)	36.9 (10.8)	34.4 (10.2)	0.262
Disease duration, mean years (SD)[b]	8.6 (7.4)	9.9 (8.5)	7.8 (6.7)	0.225
EDSS score, median (IQR)[c]	2 (1.5–5.5)	3 (2–5.5)	2 (1.5–4.5)	**0.026**
No of relapses in the past year, mean (SD)[b]	0.4 (0.6)	0.26 (0.5)	0.4 (0.7)	0.244
Treated with DMTs, *n* (%)[a]	88 (89.8%)	32 (91.4%)	56 (88.9%)	0.691
FSS, median (IQR)[c]*	5.0 (3.6–6.0)	4.6 (3.7–5.6)	5.2 (2.9–6.1)	0.449
MADRS, median (IQR)[c]	6.0 (4.0–9.0)	6.0 (4.0–9.0)	6.0 (4.0–8.0)	0.676
No of failed tests, mean (SD)[b]	1.9 (2.3)	1.5 (1.8)	2.13 (2.5)	0.051
SRT-LTS score, median (IQR)[c]	38.2 (28.2–45.4)	38.2 (32.2–46.3)	36.2 (25.4–43.4)	0.184
SRT-CLTR score, median (IQR)[c]	27.4 (19.9–39.1)	27.4 (22.1–39.1)	27.4 (19.1–38.1)	0.784
SRT-D score, median (IQR)[c]	7.7 (5.0–9.3)	7.9 (5.3–9.5)	7.5 (4.9–9.3)	0.580
SPART score, median (IQR)[c]	18.7 (15.4–23.7)	19.2 (15.9–23.8)	17.8 (13.9–21.9)	0.252
SPARTD score, median (IQR)[c]	6.3 (4.9–7.9)	6.9 (5.2–8.9)	6.3 (4.9–7.3)	**0.025**
SDMT score, median (IQR)[c]	48.4 (42.2–57.3)	49.2 (42.5–57.6)	47.4 (41.5–57.2)	0.477
WLG score, median (IQR)[c]	23.0 (18.0–26.7)	21.1 (16.9–26.9)	23.1 (18.9–26.9)	0.333
ST score, median (IQR)[c]	53.5 (41.4–63.2)	51.1 (34.6–61.7)	54.3 (47.8–63.7)	0.221
Pasat2 score, median (IQR)[c]	25.5 (8.3–34.4)	29.3 (16.9–34.2)	203.3 (4.9–34.8)	0.104
Pasat3 score, median (IQR)[c]	40.2 (27.7–49.0)	38.4 (28.9–49.6)	40.5 (23.9–46.5)	0.417

**available in 68 subjects; [a]Chi-squared; [b]t-test for independent samples; [c]Mann–Whitney U test. IQ, intelligence quotient; RR, relapsing-remitting; CP, chronic progressive; EDSS, Expanded Disability Status Scale; DMTs, disease-modifying treatments; FSS, Fatigue Severity Scale; MADRS, Montgomery and Asberg Depression Rating Scale; SRT, Selective Reminding Test; SRT-LTS, Selective Reminding Test–Long Term Storage; SRT-CLTR, Selective Reminding Test–Consistent Long Term Retrieval; SRT-D, Selective Reminding Test–Delayed; SPART, Spatial Recall Test; SPART-D, Spatial Recall Test–Delayed; SDMT, Symbol Digit Modalities Test; WLG, Word List Generation; ST, Stroop Test; PASAT-2, Paced Auditory Serial Addition Test−2 seconds; PASAT-3, Paced Auditory Serial Addition Test−3 seconds; SD, standard deviation. The bold values are the statistically significant ones ($p < 0.05$).*

automated method (QIAcube, QIAGEN). After DNA extraction, a high-resolution melting analysis (HRMA) method was used to analyze the presence of *rs6265* polymorphism, using the following primers: 5′-ACTCTGGAGAGCGTGAATGG-3′ and 5′-ACTACTGAGCATCACCCTGGA-3′ for the polymerase chain reaction (PCR) to amplify the subjects' DNA.

Statistical Analysis

Demographic and clinical characteristics were described as frequency (percentage) and mean ± standard deviation (SD). Group comparisons were assessed through the Pearson's chi-square, Student *t*, and Mann–Whitney *U* tests when appropriate. Possible predictors of the number of failed neuropsychological tests were assessed through a backward stepwise linear regression model, including as covariates BDNF genotype, sex, age, education, disease duration, disease course, number of relapses in the year before inclusion, EDSS, treatment, and premorbid IQ. $P < 0.05$ were considered as significant. Likewise, possible predictors of EDSS score were assessed through a backward stepwise linear regression model, including as covariates BDNF genotype, sex, age, disease duration, disease course, number of relapses in the year before inclusion, and treatment.

RESULTS

Ninety-eight patients were included in the analysis, 80 (81.6%) with a RR, 12 (12.3%) with a SP, and six (6.1%) with a PP course. SP and PP patients were analyzed as a whole group, named chronic progressive (CP) MS. The genetic analysis identified 35 (35.7%) patients with the BDNF *rs6265* (Val/Met) polymorphism. The main demographical and clinical characteristics of the whole sample and of the two groups (Val/Met and Val/Val) are depicted in **Table 1**. In the univariate analysis, patients with the BDNF *rs6265* polymorphism were more frequently males (48.6 vs. 24.4%, $p = 0.020$, chi-squared test), more disabled (median EDSS score 3, IQR: 2–5.5 vs. 2, IQR 1.5–4.5, $p = 0.026$, Mann–Whitney *U* test), and, marginally, older (46.7 ± 10.7 vs. 42.3 ± 10.8 years, $p = 0.064$, *t*-test for independent samples) than Val/Val patients. Eighty-eight (89.8%) patients were treated with disease-modifying therapies (DMTs) (two azathioprine; 17 interferon in its various formulations; nine glatiramer acetate; 39 dimethyl

fumarate; three teriflunomide; 10 natalizumab; one methotrexate; one cyclophosphamide; six fingolimod) (31).

As for cognitive assessment, at the univariate analysis, Val/Met patients demonstrated a trend toward a lower number of failed neuropsychological tests as opposed to Val/Val patients (1.5 ± 1.8 vs. 2.1 ± 2.5, $p = 0.051$, *t*-test for independent samples). Moreover, BDNF Val/Met polymorphism patients had higher mean adjusted score on the SPART-D (median 6.9, IQR: 5.2–8.9 vs. 6.3, IQR: 4.9–7.3, $p = 0.025$, Mann–Whitney *U* test). There were no differences in the other adjusted scores obtained in the remaining neuropsychological tests. The same was true when comparing the MADRS and FSS scores obtained by Met carriers and Val/Val homozygotes.

In the multivariable analysis, the presence of the BDNF *rs6265* Val/Met polymorphism ($B = -1.1 \pm 0.5$, $p = 0.027$) and, marginally, a higher IQ ($B = -0.6 \pm 0.03$, $p = 0.068$) were associated with a lower number of failed cognitive tests. On the other hand, higher EDSS score was associated with a higher mean number of failed neuropsychological tests ($B = 0.385 \pm 0.128$, $p = 0.003$). The *R*-square for the model was 21.1%, with an adjusted *R*-square for the overall model of 18.4%, a medium size effect according to Cohen (32) (**Table 2**).

As for disability, a higher EDSS score was associated with a CP course ($B = 3.4$, $p < 0.001$), while there was a trend toward an association with Val/Met polymorphism ($B = 0.56$, $p = 0.066$). Other variables included in the model were sex, age, disease duration, mean number of relapses in the last year, and treatment. The *R*-square for the model was 48.4%, with an adjusted R-square for the overall model of 47.3%, a large size effect according to Cohen (32) (**Table 3**).

TABLE 2 | Predictors of number of failed neuropsychological tests based on a linear regression model.

	β	*p*
EDSS	0.385	**0.003**
IQ	−0.6	0.068
BDNF *rs6265* (Val/Met) polymorphism	−1.1	**0.027**

Adjusted R-square for the model: 0.184. EDSS, Expanded Disability Status Scale; IQ, intelligence quotient; BDNF, brain-derived neurotrophic factor. Covariates that were not retained in the final model are as follows: disease course, sex, age, disease duration, treatment with disease-modifying therapies, education, and number of relapses in the last year. The bold values are the statistically significant ones (p < 0.05).

TABLE 3 | Predictors of EDSS score based on a linear regression model.

	β	*p*
CP course	3.382	**<0.001**
BDNF *rs6265 (Val/Met)* polymorphism	0.559	0.066

Adjusted R-square for the model: 0.473. EDSS, Expanded Disability Status Scale; CP, chronic progressive; BDNF, brain-derived neurotrophic factor. Covariates that were not retained in the final model are as follows: sex, age, disease duration, treatment with disease-modifying therapies, and number of relapses in the last year. The bold values are the statistically significant ones (p < 0.05).

DISCUSSION

While BDNF has been consistently associated with better cognitive performances in healthy individuals and was found to be a protective factor against memory impairment in neurodegenerative disorders (such as Alzheimer disease) (10), its role in neuroinflammatory diseases is still poorly understood. In MS, previous studies on possible relationships between BDNF and both cognitive and motor disability have reported conflicting results (17–20). In our cross-sectional multicenter study, we assessed the role of the BDNF *rs6265* polymorphism on cognitive functions and disability among MS patients.

Carriers of Met allele showed an overall better cognitive performance, failing a lower number of neuropsychological tests. The strength of this association, which was marginal at the univariate analysis, significantly increased after adjustment for well-acknowledged demographic and clinical confounders of cognitive functioning in MS (in particular age and disability, which were unevenly distributed between the two groups) (33).

Our results are in line with another study exploring the role of BDNF Val/Met polymorphism in MS on MRI parameters and cognitive performances on the PASAT, a test of information processing speed and complex attention (19). Indeed, in that study, Met carriers had both higher GM volumes and better cognitive performances than Val/Val carriers. The same protective role of BDNF *rs6265* polymorphism against brain atrophy was highlighted in other subsequent studies (18, 20). Moreover, in a recent functional-MRI study, the BDNF Val/Met polymorphism was associated with increased functional connectivity between the hippocampus and posterior cingulate cortex in comparison with Val homozygosis during retrieval phase of an episodic memory task, while the opposite was true for healthy controls (34).

In general, conversely to what has been demonstrated in the general population and neurodegenerative diseases, findings from our and the abovementioned studies suggest a protective role of BDNF Val/Met polymorphism against cognitive decline and brain atrophy in MS patients. The impact of BDNF in MS could potentially be very different from that in healthy individuals and other pathological conditions, due to differences in the pathophysiological milieu in which BDNF exerts its effects. The neuroinflammatory environment of the MS lesions contains immune cells, such as infiltrating T-cells and macrophages, as well as activated astrocytes. These cells were found to express higher BDNF mRNA levels (35–37), contributing to increased BDNF secretion. In addition, BDNF could have a dual role in the setting of neuroinflammation, depending on its concentration. For instance, neurons populating the edges of active lesions and oligodendrocytes (and their precursors) have been found to express higher levels of two different BDNF receptors, the TrKB BDNF receptor (35) and the p75 neurotrophin receptor (NTR) (38), respectively. These two receptors have different affinities for BDNF and mediate different effects of this molecule. In particular, the high-affinity TrKB receptor (active at low BDNF concentrations) mediates the signaling cascade connected with neuronal survival, while the low-affinity p75 NTR (binding with

BDNF at higher concentrations) is thought to mediate a pro-apoptotic role. Therefore, an increased production of BDNF in a neuroinflammatory milieu can have a detrimental effect, shifting the balance toward apoptosis, and neurodegeneration.

Furthermore, BDNF is known to facilitate glutamatergic synaptic transmission via mechanisms involving the N-methyl-D-aspartate receptors (39). This action has a crucial role for neuroplasticity and long-term potentiation, which are fundamental for learning and memory, and could account for the positive effect of BDNF in healthy population and neurodegenerative diseases (6, 12). On the other hand, in MS patients, LTP activation and glutamate excitotoxicity can cause oligodendrocytes and neuronal loss (40). Additionally, neuronal processes requiring the activity-dependent component of BDNF could be compromised by the constitutive presence of the immune cell-derived BDNF.

Against this background, it could be argued that the presence of abundant BDNF in an inflammatory environment could be detrimental for neuronal functions, promoting toxicity mechanisms that could enhance synaptic degeneration. Taken as a whole, these actions can hinder cognitive functioning, contributing to neuropsychological impairment.

In our study, beyond BDNF polymorphism, CI was associated with greater disability levels as measured on the EDSS. This finding is consistent with the existing literature, showing that age and disability levels are the main drivers of neuropsychological dysfunction in MS (33).

As for motor disability, while a potential negative effect of Val/Met polymorphism emerged in the univariate analysis, in the multivariable analysis, the only significant predictor of higher EDSS score was the CP course of the disease. The neutral role of the BDNF *rs6265* polymorphism on disability in MS was also evident in a large cross-sectional study conducted in Norway including 2,149 MS patients (21). The absence of a significant relationship between BDNF polymorphism and motor disability can be due, at least in part, to the differential expression of BDNF in the CNS. Indeed, greater expression of BDNF has been reported in brain regions involved in learning and memory, such as the hippocampal formation and the prefrontal cortex, where the anatomical effect of Val66Met polymorphism is most apparent (8, 11, 12). It must be noted that other regulating factors, which were not assessed in our study, such as epigenetic mechanisms and DNA methylation, can modulate the effects of BDNF polymorphism. In a recent study on 209 MS patients, while the presence of Val/Met polymorphism was not linked to disability accumulation, a lower BDNF gene DNA methylation, and therefore, higher gene expression and BDNF secretion, was associated to a higher risk of reaching EDSS 6.0 (41). Whether higher BDNF expression is directly responsible of disability worsening or represents an ineffective compensatory attempt needs to be clarified.

In interpreting the study findings, a few limitations should be considered. The sample size was relatively small. In the univariate analysis, Met carriers were more frequently males, more disabled, and, marginally, older than Val/Val homozygotes, reflecting a possible sampling bias. These differences can account, at least in part, for the marginal association between BDNF polymorphism and motor disability, which disappeared in the multivariable model. On the other hand, older age and greater disability in Met carriers are expected to increase the proportion of CI in this group: in this respect, as commented above, our findings seem to reinforce the hypothesis of a protective effect of BDNF polymorphism against CI in MS. Moreover, data on MRI evaluations are lacking, as well as measurement of actual levels of BDNF at the time of clinical and neuropsychological evaluations. Finally, since genetics has influence during the course of the disease, the cross-sectional design prevented the assessment of a possible longitudinal effect of BDNF polymorphism on study outcomes.

Despite these limitations, our results suggest a protective role of BDNF Val66Met polymorphism against CI in MS patients, possibly reflecting a detrimental effect of increased BDNF concentration in a neuroinflammatory environment. These preliminary findings indicate that BDNF and its polymorphism may represent a potential biomarker for susceptibility and severity of CI in MS, as well as a possible therapeutic target of pharmacological interventions for neuropsychological dysfunction. Further studies are needed to confirm our findings on larger populations, with longitudinal MRI and clinical evaluations.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository and accession number can be found here: Harvard Dataverse, accession link: https://dataverse.harvard.edu/loginpage.xhtml?redirectPage=%2Fdataset.xhtml%3FpersistentId%3Ddoi%3A10.7910%2FDVN%2FOVA6P9. Anonymized data will be shared on reasonable request from a qualified investigator.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Local Ethic Committes. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EPo, AB, EPr, SS, and MA developed the original concept of the study, developed the analysis plan, and did the manuscript writing. EPo, LR, LP, MF, and GZ contributed to clinical data acquisition. CN, BG, and NL contributed to data acquisition and performed the neuropsychological assessment. BN and SB developed and performed laboratory analyses. All authors reviewed and commented on drafts of the protocol and paper. All authors read and approved the final manuscript.

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BRIEF RESEARCH REPORT
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Brain Volume and Perception of Cognitive Impairment in People With Multiple Sclerosis and Their Caregivers

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Background: Cognitive impairment (CI) is common in people with multiple sclerosis (pwMS). The assessment of CI is based on neuropsychological tests and accurate anamnesis, involving the patients and caregivers (CG). This study aimed to assess the complex interplay between self-perception of CI, objective CI and the brain atrophy of MS patients, also exploring the possible differences with CI evaluated by caregivers.

Methods: Relapsing pwMS were enrolled in this study. Subjects underwent neuropsychological examination using the Brief Cognitive Assessment for Multiple Sclerosis (BICAMS) and evaluation of self-reported cognitive status using the patient-version of the Multiple Sclerosis Neuropsychological Questionnaire (p-MSNQ). Depression and anxiety were also evaluated using the Back Depression Inventory-version II (BDI-II) and Zung Anxiety Scale. Brain MRI images were acquired and brain volumes estimated. For each patient that was enrolled, we spoke to a caregiver and collected their perception of the patient's CI using the MSNQ- Caregiver version.

Results: Ninety-five MS subjects with their caregivers were enrolled. CI was detected in 51 (53.7%) patients. We found a significant correlation ($p < 0.001$) between BICAMS T scores and lower whole brain (Rho = 0.51), gray matter (Rho = 0.54), cortical gray matter (Rho = 0.51) volumes and lower p-MSNQ (Rho = 0.31), and cg-MSNQ (Rho = 0.41) scores. Multivariate logistic regression showed that p-MSNQ is related to a patient's anxiety to evaluate by Zung Score ($p < 0.001$) while cg-MSNQ to patient's brain volume ($p = 0.01$).

Conclusion: Our data confirm that neuropsychological evaluation results are related to the perception of CI and brain volume measures and highlight the importance of the caregiver's perception for cognitive assessment of pwMS.

Keywords: multiple sclerosis, cognitive impairment, caregiver, brain volume, patients

INTRODUCTION

Cognitive dysfunctions are frequent and represent a major concern for people living with multiple sclerosis (pwMS). Several studies estimated that the prevalence of cognitive impairment (CI) among pwMS ranges between 40 and 70%, occurring in subjects with different clinical course and MS features, early as in more advanced stages of the disease (1). In the last few years, growing attention has been paid to the evaluation of CI in MS, also because of the impact of this invisible but heavy symptom on several aspects of patients' lives. For this reason, numerous neuropsychological assessments have been proposed, including rapid screen tools principally useful in a clinical setting and self-reported questionnaires aimed to evaluate the perception of patients' cognitive functioning (2). The Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) is used in clinical settings, due to its rapidity of administration and the evaluation of principle cognitive domains affected by MS (3–5). The Multiple Sclerosis Neuropsychological Questionnaire (MSNQ), including a patient and caregiver (CG) version, has emerged as the most used tool worldwide for evaluating the perception of patients' CI (6). The relationship between the objective and perceived CI is notoriously extremely complicated and is potentially influenced by MS-related structural brain damage (7–9) as well as several others factors (10, 11) among which are also mood disorders (7, 8). Based on these considerations, this study aims to evaluate the complex interplay between CI of pwMS and the perception of cognitive functioning reported by patients and their CG, also exploring the possible relationships with brain volume measurements.

METHODS

Participants

Patient Recruitment

Consecutive relapsing remitting pwMS were enrolled at the Multiple Sclerosis Center of Binaghi Hospital, ATS Sardegna. Exclusion criteria were: (i) exposure to corticosteroid or occurrence of clinical relapse in the previous 30 days; (ii) change in disease modifying therapy in the previous 6 months; (iii) presence of other chronic comorbidities; (iv) use of drugs or substances with a psychotropic effect; (v) contraindications to underwent MRI; (vi) presence of a physical disability that did not allow the neuropsychological evaluation (i.e., blindness).

All included MS patients underwent a clinical, neuropsychological, and brain MRI examination in the same week. Demographics and clinical MS features [gender, age, education, disease duration, and level of disability, assessed by Expanded Disability Status Scale (EDSS) score] (12) were also collected.

Caregiver Recruitment

For each enrolled patient, a caregiver was included. Caregivers were classified based on the relationship with the patients. Thus, the CG version of MSNQ (13) was administrated to the participants to capture their views on the patient's cognitive functioning. Informed consent was obtained from all participants (pwMS and CG) included in the study, which was approved by the local ethics committee.

Neuropsychological Assessment

The cognitive functions of the included patients were evaluated using the Italian version of the BICAMS battery (5) with implemented normative values for the Italian population and corrections for sex, age, and years of education (14). The BICAMS battery includes the Symbol Digit Modalities Test (SDMT) for evaluating the information processing speed, the California Verbal Learning Test (CVLT-II) for evaluating verbal learning and memory, and the Brief Visual Memory Test (BVMT) for evaluating visual learning and memory (5). In our study, according to the Italian validation process of the BICAMS battery, we included the total number of correct responses in 90 seconds for SDMT, the total number of words recalled over five learning trials (Total Learning, TL) for CVLT-II, and total recall score across the three trials.

According to the authors' definition, each test was classified as altered if the T Score was below 35 points. Thus, the self-perception of the CI of the patients was evaluated using the p-version of MSNQ (13).

The T score of any BICAMS tests was reported for each included patient, the mean T score of all BICAMS tests and the sum of BICAMS tests scored below 35 T score (number of altered tests). Finally, depression and anxiety were evaluated using the BDI-II and the Zung Anxiety Scale (15, 16).

MRI Acquisition

Brain MRIs were acquired using a Magnetom Avanto Scanner (Siemens, Enlargen) at 1.5 T. The MRI protocol included the following sequence: 3D T1-Magnetization Prepared Rapid Acquisition Gradient-Echo (MPRAGE): echo time (TE): 2.37 ms; repetition time (TR): 1,730 ms; inversion time (TI): 1,050 ms; field of view (FOV): 244 mm; voxel size: 1 × 1 × 1 mm, (176 contiguous slices). A dual-echo, turbo spin-echo sequence (repetition time/echo time 1/echo time 2 5 2,075/30/90 ms, 256 3256 matrix, one signal average, 250-mm field of view, 50 contiguous 3-mm slices) yielding proton density–weighted and T2-weighted images oriented to exactly match the MPRAGE image acquisition. Brain parenchyma volumes were measured on T1W gradient echo images using the cross-sectional version of SIENA (structural image evaluation using normalization of atrophy) software, SIENAX (part of FSL 4.0: http://www.fmrib.ox.ac.uk/fsl/), and a previously described method to estimate the overall brain volume, normalized for head size. MRI analysis allowed us to obtain normalized whole brain volume (WB), normalized gray matter volume (GM), normalized white matter

Abbreviations: BICAMS, Brief International Cognitive Assessment for Multiple Sclerosis; BVMT, Brief Visual Memory Test-Revised; CFs, Cognitive Functions; cgMSNQ, Multiple Sclerosis Neuropsychological Questionnaire- caregiver version; CGs, Caregivers; CI, Cognitive impairment; CVLT, California Verbal Learning Test; MS, Multiple Sclerosis; pMSNQ, Multiple Sclerosis Neuropsychological Questionnaire-patient version; SDMT, Symbol Digit Modalities Test; WB, whole brain; WM, whole white matter; GM, whole gray matter; cGM, cortical gray matter.

volume (WM), and normalized cortical gray matter volume (cGM). T1 hypo-intense lesion refilling was performed as previously described (17, 18). The radiologist was blinded to the results of the cognitive and neurological evaluation.

Statistical Analysis

All statistical analyses were performed using SPSS for Mac version 20.0 (SPSS Inc., Chicago. IL, USA). First, descriptive analysis was performed. Next, we used the Shapiro-Wilks and Kolmogorov-Smirnov for testing the normality of variables. Based on normal distribution evaluation, we used a parametric or non-parametric test to evaluate the correlation between the variables evaluated. the relationship of BICAMS Tests Results with brain volumes was assessed by Pearson or Spearman test. Analogously, the relationship of p-MSNQ and cg-MSNQ scores with BICAMS Tests Results and brain measurements were evaluated. Thus, regression analyses were performed to evaluate which factors influence p-MSNQ and cg-MSNQ scores, included in each model as dependent variable, also controlling for BDI-II and Zung Anxiety scores. Moreover, we performed a collinearity diagnostic test regarding the linear regression. For all assays, the statistical significance was set at $P < 0.05$.

The results were filtered using the Benjamini-Hochberg procedure for FDR correction (FDR < 0.05). The test of the collinearity of variables also included multivariate linear regression analysis.

RESULTS

The sample included 95 MS relapsing remitting patients (68/95; 71.6% female). Mean values for age and disease duration were, respectively, 43.65 (SD: 11.9) and 12.1 (SD: 7.8) years, while the median EDSS score was 2.0 (IQR: 0–5.5). For each MS patient, a caregiver was included. Of these, 62 were partners (65.2%), and 33 family caregivers (34.8%). **Table 1** shows the demographic and clinical features of participants included in the study. CI, defined by at least one impaired test at the BICAMS assessment, was relieved in 51 (53.7%) of patients.

We found a significant correlation of mean BICAMS T scores with measurements of WB (Rho = 0.50), GM (Rho = 0.545), and cGM (Rho = 0.517), ($p < 0.001$), as shown in **Table 2**.

As shown in **Table 3**, the relationship of mean BICAMS T scores with p-MSNQ (Rho = 0.31 $p < 0.01$) and cg-MSNQ (Rho = 0.41; $p < 0.001$) is also observed. In addition, the perception of CG, as indicated by cg-MSNQ score, inversely correlates with WB (Rho = −0.495), GM (Rho = −0.554) and cGM (Rho = −0.563) volumes. No significant correlation was found between the patient's point of view, indicated as p-MSNQ scores, and brain volume measurements (**Table 3**).

A multivariate linear regression model was also performed. First, we included as dependent variable p-MSNQ founding a significant association of p-MSNQ scores with anxiety evaluated by Zung scores ($P = 0.001$) also controlling for BDI results, mean of BICAMS T scores, and brain volume (**Table 4A**). Moreover, we performed another analysis including the cg-MSNQ score as a dependent variable, highlighting a relationship with the patients' lower brain volume ($p = 0.01$) with no significant relationship with depression, anxiety, and BICAMS results (**Table 4B**). The variance inflation factor (VIF) values and the condition index results were not indicative of collinearity for variables included in multivariate linear regression analysis.

TABLE 1 | Demographic and clinical features of pwMS and their caregivers.

	Pw MS (95)	CG (95)
Female	68 (71.6%)	60 (63.1%)
Age (mean ± sd) years	43.65 ± 11.9	49.5 ± 10.2
Education (mean ± sd) years	13 ± 3.5	12.3 ± 4.4
MS duration (mean ± sd)	12.1 ± 7.8	
EDSS score Median (IQR)	2.0 (0–5.5)	
Whole Brain volume ml (mean ± sd)	1434.55 ± 99.68	
White matter ml (mean ± sd)	673.66 ± 37.30	
Gray matter ml (mean ± sd)	760.88 ± 78.58	
Cortex ml (mean ± sd)	594.51 ± 62.00	
SDMT T scores (mean ± sd)	42.02 ± 11.17	
CVLT T scores (mean ± sd)	44.30 ± 13.77	
BVMT T scores (mean ± sd)	48.11 ± 12.44	
BICAMS T scores (mean ± sd)	44.9 ± 10.57	

TABLE 2 | Correlations of brain volume with T scores at BICAMS assessment.

	N # failed tests	SDMT T scores	CVLT T scores	BVMT T scores	BICAMS Mean T scores
Whole brain	−0.423**	0.495**	0.389**	0.456**	0.501**
White matter	−0.137	0.217*	0.157	0.181	0.186
Gray matter	−0.501**	0.523**	0.420**	0.494**	0.545**
Cortex	−0.478**	0.485**	0.410**	0.461**	0.517**

*$p < 0.01$.
**$p < 0.001$.

TABLE 3 | Correlations of p-MSNQ and cg-MSNQ scores with BICAMS results and brain volume measurements.

	p-MSNQ scores	cg-MSNQ scores
N# failed tests	0.168	0.401**
SDMT T scores	−0.349**	−0.451**
CVLT T scores	−0.300**	−0.328**
BVMT T scores	−0.217*	−0.328**
BICAMS T scores	−0.317*	−0.416**
Whole brain	−0.131	−0.495**
White matter	−0.197	−0.116
Gray matter	−0.072	−0.554**
Cortex	−0.004	−0.563**
Zung scores	0.593**	0.232
BDI scores	0.225	0.008

*$p < 0.01$.
**$p < 0.001$.

TABLE 4 | Multiple regression analyses.

A: Dependent Variable: p-MSNQ Scores			B: Dependent Variable: cg-MSNQ Scores		
Independent variables	**Standardized beta**	***p*-value**	**Independent variable**	**Standardized beta**	***p*-value**
Bdi scores	−0.100	ns	BDI scores	−0.060	ns
Zung scores	0.622	**0.001**	Zung scores	0.129	ns
Bicams mean T score	0.087	ns	BICAMS mean T score	−0.203	ns
Whole brain	−0.191	ns	Whole brain	−0.429	**0.01**

Multiple linear regression analysis was used to examine the relationship between p-MSNQ and cg-MSNQ scores, included in the model as a dependent variable, with BDI-II, Zung, BICAMS T scores, and whole brain volume (independent variables).
Relationship of the number of p-MSNQ and cg-MSNQ scores with depression, anxiety, mean BICAMS T scores, and brain volume measurements. Bold values are statistically significant with a $p < 0.05$.

DISCUSSION

Our results confirmed the universally recognized role of MRI analysis as principal biomarkers of cognitive functions in MS (19). The present study also found a strong correlation between the volumes of the whole brain, gray matter, and cortical volume with the results of cognitive tests.

As observed in other neurological diseases, MRI measurements are not enough to fully explain cognitive deficits in MS (20). In recent decades several studies have aimed to investigate how other factors play a role (21). Among these factors, cognitive reserve, several demographic, clinical, mood disorders, and social variables could act as moderators (22, 23). However, brain volume measures showed a strong and significant relationship with all cognitive functions evaluated and the global cognitive status of MS patients.

The other aim of our study was to explore the reliability perception of cognitive impairment in Multiple Sclerosis. The data show that caregiver perception is more strongly correlated to the objective cognitive performance of people with MS than their self-judgment. In other neurological pathologies such as neurodegenerative diseases, it is a common observation that the cognitive ability self-perception of the patient is less accurate than caregiver perception (24–26).

As previously described, cognition self-judgment is often more conditioned by mood disorders such as depression and anxiety than by objective cognitive deficit (27). A severe mood disorder could interfere with both anamnestic interview and neuropsychological evaluation (28), complicating the estimation of cognitive functions and leading to overestimation of the impairment of cognitive abilities. As in our cohort, the perception of cognitive functioning reported by patients appeared to be related to anxiety in a model controlled for brain volume and the results of neuropsychological assessment (28).

Several other previous studies have evaluated the reliability of cognitive function self-judgment compared to caregiver evaluation and relationship with a mood disorder. O'Brien et al. found that p-MSNQ correlated with depression as assessed by BDI, while cg-MSNQ was independent from mood disorders, but was correlated with cognitive impairment as assessed by an extended neuropsychological battery (29). Another previous study indicated that in MS patients, after controlling for demographic variables, anxiety was a significant predictor of p-MSNQ scores, while the patients' point of view did not correlate with the results of neuropsychological examination (30). A recent study, conducted on the Danish MS population confirmed that the p-MSNQ version measures these items more than the cognitive abilities of the patients (31). These previous studies are in line with our results which confirm that the patient's self-assessment of their cognitive functions is related more to the characteristics of their mood than to objective evaluation.

Interestingly, the relationship between caregiver perception of a patient's cognition and patients' brain volume emerged as an unexpected result of our study. The perception of CI reported by the caregivers shows a strong correlation with patient brain volume measures, whole brain, and gray matter, while there is no correlation between p-MSNQ and brain atrophy. In the multivariate analysis, the cg-MSNQ scores were also related to patients' brain volume, even after controlling for depression BDI-II scores, anxiety Zung scores, and neuropsychological test results. As previously described (28), the caregiver's evaluation of the patient's cognitive functions is based on multiple issues such as skills in daily life, detailed knowledge of the premorbid level of cognitive skills, and the social context of the patient. Consequently, our data support the hypothesis that the perception of the caregiver is related to the effective cognitive functioning of the patient as documented by the strong correlation with the brain volume confirmed also in the multivariate analysis. Thus, caregiver evaluation of cognitive functioning in MS emerges as related to brain volume as an indication of structural damage. The absence of a correlation between patient self-evaluation and brain volume measure could be explained by processes such as the influence of mood disorders, especially anxiety, on self-evaluation and a lack of insight about impairment in patients with severe brain atrophy.

Recently, several studies on metacognition have also contributed to the understanding of the complex interplay that regulates the perception of cognitive disorders in MS (32). These findings are in line with our results and point to the role of mood disorders in self-perception of cognitive impairment in people with MS. Our study also adds the significant relationship between the caregiver's point of view, cognitive measures and brain volume as the main biomarker of cognitive impairment.

Our study shows several limitations. First, the limited number of pwMS included in the research could influence the application of the results. Second, the MRI biomarkers included only

the brain volume measurements while also other radiological features are associated with CI in MS as white matter total lesion load that was not included in the present study. Furthermore, even if using appropriate statistical tests, given the limited size of the sample, it is not possible to exclude errors due to the association between the evaluated measures.

CONCLUSIONS

In conclusion, our study confirmed the well-known importance of MRI volumetric measurements as biomarkers of CI in MS based on the relationship with cognitive results. Furthermore, the caregiver's point of view appears to be stronger related to neuroradiological biomarkers of cognitive deficit and neuropsychological assessment test results rather than patient self-evaluation.

This data suggests the importance of including the caregivers' judgment in the anamnestic evaluation of pwMS undergoing neuropsychological assessment. Further studies are needed to better evaluate what tools to use in a clinical setting to capture both MS patients' and caregivers' perceptions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Cagliari. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GF and LL participated in the design of the study and drafted the manuscript. ECa, MA, MF, and AC carried out the neuropsychological evaluation and performed the statistical analysis and drafted the manuscript. JF and GC revised the manuscript for important intellectual content and performed the statistical analysis. FC and MB acquired MRI images. ECo helped draft the manuscript and revised it critically for important intellectual content. All authors read and approved the final manuscript.

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Insights on the Relationship Between Hippocampal Connectivity and Memory Performances at the Early Stage of Multiple Sclerosis

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While memory impairment in multiple sclerosis (MS) is known to be associated with hippocampal alterations, whether hippocampal networks could dynamically reorganize as a compensation mechanism is still a matter of debate. In this context, our aim was to identify the patterns of structural and functional connectivity between the hippocampus and the rest of the brain and their possible relevance to memory performances in early MS. Thirty-two patients with a first episode suggestive of MS together with 10 matched healthy controls were prospectively explored at baseline, 1 and 5 years follow up. They were scanned with MRI and underwent a neuropsychological battery of tests that included the Selective Reminding Test and the Brief Visual Memory Test Revised to assess verbal and visuo-spatial memory, respectively. Hippocampal volume was computed together with four graph theory metrics to study the structural and functional connectivity of both hippocampi with the rest of the brain. Associations between network parameters and memory performances were assessed using linear mixed-effects (LME) models. Considering cognitive abilities, verbal memory performances of patients decreased over time while visuo-spatial memory performances were maintained. In parallel, hippocampal volumes decreased significantly while structural and functional connectivity metrics were modified, with an increase in hippocampal connections over time. More precisely, these modifications were indicating a reinforcement of hippocampal short-distance connections. LME models revealed that the drop in verbal memory performances was associated with hippocampal volume loss, while the preservation of visuo-spatial memory performances was linked to decreased hippocampal functional shortest path length. In conclusion, we demonstrated a differential impairment in memory performances in the early stages of MS and an important interplay between hippocampal-related structural and functional networks and those performances. As the structural damage increases, functional reorganization seems to be able to maintain visuo-spatial memory performances with strengthened short-distance connections.

Keywords: multiple sclerosis, clinically isolated syndrome, memory, hippocampus, functional connectivity, structural connectivity, graph theory

INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating, and neurodegenerative disorder of the central nervous system. The progression of the disease is typically characterized by physical disability such as motor or sensory symptoms that are related to the recurrence of inflammatory attacks. In addition to those symptoms, 40–70% of MS patients also experience cognitive impairments (1) which can appear early in the course of the disease, even at the stage of clinically isolated syndrome (CIS), the first episode suggestive of further MS. It is now accepted that cognitive impairment in MS is negatively associated with quality of life and strongly impacts vocational status and rate of unemployment (2).

Different cognitive domains can be impaired in the context of MS such as memory, information processing speed or executive functions, with some inter-patient variability (1). Amongst these different domains, memory is one of the most consistently impaired with approximately half of the patients concerned (3). Nevertheless, the pathophysiology of memory impairment in MS is still a matter of debate and should be clarified in order to target therapeutic strategies including specific cognitive rehabilitation programs.

Most studies now agree on hippocampal involvement. Post-mortem pathological studies and *in vivo* MRI studies have pointed toward a vulnerability of the hippocampus to the inflammatory environment associated with MS. Indeed, post-mortem studies of MS patients have reported hippocampal demyelination, neural loss, and a decreased expression of neuronal proteins, ultimately leading to tissue atrophy (4, 5). In addition, *in vivo* MRI studies have also been able to capture such structural damages in terms of hippocampal volume loss, alteration of microstructural metrics or modification of structural connectivity: all of them showing some degree of correlation with memory impairment in MS patients (6–10).

However, whether functional reorganization could help compensate such damages to mitigate memory deficit is a matter of intense debate. Indeed, functional MRI (fMRI) can now be used to explore non-invasively the functional activity of the brain during a task or at rest, in the so-called resting state fMRI (rs-fMRI). From rs-fMRI, Schoonheim et al., proposed that a compensatory mechanism could be put into play in the form of a functional reorganization of networks to compensate for structural alterations induced by the disease and to mitigate clinical deficits (11). This theory would explain a delay in cognitive impairment appearance after the onset of the disease and is important because, if true, it could justify the "stimulation" of such alternate networks through specific rehabilitation and training programs. However, different reports provided conflicting data with respect to this model. Some authors have reported a decrease in functional connectivity in memory impaired compared to preserved MS patients which could be interpreted as a lack of compensation (6, 12). Comparable results were reported in an activation study where cognitively preserved patients showed an increase in activation of hippocampal memory system compared to healthy controls when performing a memorization task, while cognitively impaired patients showed less activation (13). Differently, an increase in functional connectivity among core part of the default mode network (6, 14) and between the right hippocampus and frontal areas (7) was associated with loss of cognitive efficiency rather than with preserved functions. Several limitations could explain such conflicting results; the most important being the cross-sectional designs of all these studies, with MS patients at different stages of the disease and without joined analyses of structural and functional metrics.

In this context, our aim was to identify the patterns of structural and functional connectivity between the hippocampus and the rest of the brain and their possible relevance to maintain memory performances in MS. We hypothesized that functional reorganization could compensate for structural damage, allowing a delay in memory impairment appearance.

To explore this question, we used a multimodal approach, combining *in vivo* structural measures—i.e., hippocampal volume and structural connectivity—and functional measures—i.e., rs-fMRI connectivity. We took advantage of a prospective longitudinal cohort of patients—and matched healthy controls—explored at the early stage (CIS), and followed at 1 and 5 years with an extensive MRI protocol and a large neuropsychological battery including tests to assess verbal and visual memory. This longitudinal setting from the beginning of the disease was unique to observe how memory impairment evolves during the pathology course and how the structural and functional connectivity of the hippocampus are linked to this evolution.

MATERIALS AND METHODS

Population

A prospective cohort of 32 patients who experienced a first episode suggestive of MS was recruited, <6 months after the episode. All participants provided an informed written consent, and an ethical committee approved the study (SCI-COG, ClinicalTrials.gov Identifier: NCT01865357). Inclusion criterion was to present with at least two clinically silent cerebral lesions characteristic of MS on fast fluid-attenuated inversion recovery (FLAIR) images. As for exclusion criteria, they included age below 18 years, inability to undergo MRI, history of other neurological or psychiatric disorders, MS relapse within 2 months prior to screening, corticosteroid pulse therapy within 2 months prior to screening, and severe depression [Beck Depression Inventory (15) >27]. Ten healthy controls matched for age, sex, and educational level were also included. All MS patients and healthy controls underwent a neuropsychological assessment at baseline as well as at a year 1 and year 5 follow-up. The Expanded Disability Status Scale (EDSS) scores were determined for patients at the three time points by expert neurologists and conversion (or not) to MS was judged according to 2017 McDonald criteria (16). Patients also underwent an MRI scan at the three time points, while healthy controls were only scanned at baseline and at the 5-year follow-up.

Neuropsychological Assessment

Episodic memory efficiency was assessed by two different tests: the Selective Reminding Test (SRT) (17), to evaluate

episodic verbal memory performances (three sub-scores: SRT-LTS = long-term storage; SRT-CLTR = consistent long-term retrieval; SRT-DR = delay recall) and the Brief Visual Memory Test Revised (BVMT-R) (18), to evaluate episodic visuospatial memory performances (two sub-scores: BVMTR = learning; BVMTR-DR = delayed recall). All participants also underwent a comprehensive neuropsychological battery of tests. In order to account for practice effects (test-retest effect), we compared patients' scores with healthy controls' scores at each time point (baseline, 1- and 5-year follow-up) by using *Z*-scores.

MRI Acquisition

Imaging was performed using 3 Tesla MRI systems (Achieva TX system, Philips Healthcare, Best, The Netherlands; Signa, GE Healthcare, Discovery MR 750w, Milwaukee, Wisconsin). Structural images were acquired with a 3D T1-weighted sequence using magnetization prepared rapid gradient echo (MP-RAGE) imaging (TR = 8.2 ms, TE = 3.5 ms, TI = 982 ms, $\alpha = 7°$, FOV = 256 mm, voxel size = 1 mm^3, and 180 slices) as well as a 2D FLAIR sequence (TR = 11,000 ms, TE = 140 ms, TI = 2,800 ms, FOV = 230 mm, 45 axial slices, and 3-mm thick). Diffusion images were acquired with a diffusion tensor echo-planar-imaging pulse sequence (TR = 11,676 ms, TE = 60 ms, FOV = 230 mm, voxel size = 1.6 mm^3) in 21 non-colinear directions at b = 1,000 s/mm^2, and with one b = 0 s/mm^2. Finally, resting-state functional images were acquired with a whole-brain T2*-weighted echo-planar imaging (EPI) sequence (250 volumes, 40 axial slices, TR = 2,200 ms, TE = 30 ms, voxel size = 3 mm^3). The first four volumes of the functional run were removed to reach signal stability.

Structural Preprocessing and Parcellation

The Lesion Segmentation Tool (LST) version 2.0.15 of SPM12 (http://www.applied-statistics.de/lst.html) was used to segment MS lesions on FLAIR data. Lesions were further manually corrected by two blinded experts. In order to prevent brain tissue segmentation from being biased by lesions, those masks of segmented lesions were used to apply a lesion-filling algorithm to the T1-weighted images. Whole-brain, total white-matter, gray-matter and hippocampal volumes were calculated using the volBrain system (https://volbrain.upv.es/). The segmentation procedure consists first of denoising and inhomogeneity correction, after which volumes are affine registered to the Montreal Neurological Institute (MNI) space. To control for variations in head size, each volume was assessed as a fraction of total intracranial volume (TIV). Subsequently, FreeSurfer (v5.3) image analysis suite (http://surfer.nmr.mgh.harvard.edu) was used to preprocess structural data and separate them into parcels using a custom-made atlas based on Destrieux cortical atlas (19). The latter consists of a parcellation originating from the division of the neocortex into gyral and sulcal regions, both being delineated by the curvature value of the surface. In addition, deep gray matter structures (i.e., pallidus, accumbens, putamen, caudate, and amygdala), the cerebellar cortex and the ventral diencephalon, were also included as parcels. At the end, we obtained a custom-made atlas which included 83 parcels per hemisphere. This parcellation was used to compute the structural connectivity between both hippocampi and each individual parcel (see below).

DTI Preprocessing

Diffusion data were preprocessed using the Oxford Center for Functional MRI of the Brain (FMRIB) Software Library (FSL, version 5.0.9; fsl.fmrib.ox.ac.uk/fsl) and MRtrix3 software (20) was used for diffusion-weighted tractography. We first corrected for motion artifacts and eddy current distortions. Next, fiber orientation distributions were calculated using the constrained spherical-deconvolution algorithm (21). About 10 million whole-brain streamlines were subsequently generated using the five-tissue-type segmented T1 image and the anatomically constrained tractography (20). These streamlines were cropped at the gray matter–white matter interface and further filtered to about 2 million using the spherical-deconvolution informed filtering of tractograms (22) to reduce reconstruction bias and improve biological plausibility. Finally, T1-weighted images were registered to diffusion images (b0 image as a reference) by a rigid registration followed by a non-rigid registration of the T1-weighted image to the subject's b0 space using ANTs software (23). Following this registration, the previously obtained streamlines were mapped into the 166 nodes (83 per hemisphere) of the custom-made atlas and a structural connectivity 166 × 166 matrix was computed. Each element of the matrix represents the number of streamlines between two regions normalized by the total number of streamlines for each participant, accounting for region size. Structural connectome matrices for patients are displayed in **Supplementary Figure 1**.

fMRI Preprocessing

fMRI pre-processing of images was performed using publicly available software (SPM12, FSL) following the same procedure as the one used by Yeo et al. (24). The 4 first scans of all participants were removed to reach signal stability. First, slice acquisition-dependent time shifts between volumes were compensated for. Second, head motion was corrected using rigid body translation and rotation and 6 parameters were extracted. Next, constant offset and linear trend over each run were removed and a low-pass filter was applied (0.08 Hz). Finally, the whole brain mean signal, the mean signal within the white matter and the mean signal within the ventricles were regressed out, together with the 6 motion parameters extracted during the previous step and their temporal derivatives. This last regression step aims at minimizing non-neuronal signal contributions, such as respiration-induced signal fluctuations. fMRI sequences were registered to the 3D T1 sequences with a boundary-based procedure and further visually checked. In order to analyze the blood-oxygen level dependent (BOLD) signal of the pre-processed volumes, a region-based approach was chosen. The parcellation is detailed in "structural preprocessing" section. For each parcel, the average of the BOLD time course signal of voxels belonging to this parcel was computed. Pairwise Pearson correlations between the BOLD signal of each region with all the remaining 165 regions were computed, resulting in a 166 × 166 functional connectivity matrix for each subject. Finally, a Fisher's *Z*-transformation was applied to the correlation matrices to improve normality.

Functional connectome matrices for patients are displayed in **Supplementary Figure 2**.

Connectivity Metrics

Network analysis was performed using the Brain Connectivity Toolbox (http://www.brain-connectivity-toolbox.net) (25). In order to study the structural and functional connectivity of both hippocampi with all other brain regions we focused on four metrics, coming from graph theory: strength and betweenness centrality to represent centrality properties, the average shortest path length (SPL) showing integration properties, and the clustering coefficient representing segregation properties. The strength of a node (e.g., the right or left hippocampus) is the sum of all connections it possesses with the rest of the brain. The average SPL of a node is the mean of all shortest paths between this node and all the others. The shortest path between two nodes is defined as the inverse of the sum of all connections constituting the shortest path between the two nodes. The betweenness centrality of a node is the fraction of all shortest paths in the network (i.e., the whole brain) that contain this node. Nodes with high values of betweenness centrality participate in a large number of shortest paths and thus represent the core of the network. The clustering coefficient is the fraction of a node's neighbors that are neighbors of each other. Nodes with high values of clustering coefficient are surrounded by other nodes which altogether form a cluster. We computed a mean of both right and left hippocampi for each connectivity metric.

Statistical Analysis

Statistical analyses were performed using R software (version 3.4.2, https://www.r-project.org) and SPSS 23.0 (SPSS, Chicago, IL, USA). Shapiro–Wilk test was used to test for normality of distribution. Depending on the distribution of our variables either parametric or non-parametric tests were used.

The evolution of cognitive variables over time was evaluated using paired Student *t*-tests and Wilcoxon tests depending on the distribution of each variable and significant *p*-values were extracted after Bonferroni's correction for multiple comparison.

For the evolution of MRI variables, in order to take into account possible confounding factors, we analyzed age-, sex-, education-, and scanner-standardized residuals of MRI metrics which were compared between baseline and year 5 in controls, and at each of the three time periods (baseline/year 1, baseline/year 5, and year 1/year 5) in patients. Paired Student *t*-tests and Wilcoxon tests were used depending on the distribution of residuals and significant *p*-values were extracted after Bonferroni's correction for multiple comparison.

To evaluate the link between patients' scores in memory tests and MRI metrics over time (i.e., baseline, year 1 and year 5 follow-up), we fitted linear mixed effects (LME) models with a random intercept term calculated for each patient. For each sub-score of the two memory tests, we fitted four LME models (one for each MRI metric significantly altered over time). Cognitive *z*-scores were the dependent variables and age-, sex-, education-, and scanner-standardized residuals of altered network measures were the predictor variables. The predictive power of each model was assessed using the Bayesian information criterion (BIC). The estimate of each random effect was further extracted together with the associated *p*-value after Bonferroni's correction for multiple comparison.

RESULTS

In this study we observed how memory performances evolve in the course of MS, since its onset, and how hippocampal volume together with hippocampal structural and functional connectivity can be linked to this evolution.

Patients Demographic, Clinical and Conventional MRI Characteristics

This study included 32 patients and 10 healthy controls whose characteristics were matched.

EDSS scores did not change significantly between baseline and year 1 ($p = 0.798$), nor between year 1 and year 5 ($p = 0.086$) but did increase significantly between baseline and year 5 ($p < 0.05$) (**Table 1**). T2 lesion volumes, on the other hand, did not differ significantly between baseline and year 1 ($p = 0.784$), nor between baseline and year 5 ($p = 0.065$), but did increase significantly between year 1 and year 5 ($p < 0.001$) (**Table 1**). Interestingly, whole-brain volume significantly decreased 1 year after the disease onset ($p < 0.05$). We found that this was mainly driven by alterations of white matter whose mean volume significantly decreased at the 1-year ($p < 0.01$) and at the 5-year ($p < 0.05$) follow-up while gray matter did not change significantly.

Memory Performances of Patients at Baseline, Year 1 and Year 5

Figure 1 reports patients *z*-scores to each sub-item of the two memory tests performed (SRT and BVMTR). Verbal and visuospatial memory performances were differentially affected in patients over time.

The LST was the only sub-item of both tests which did not show a significant impairment over time (**Figure 1A**). Indeed, the CLTR sub-item of the SRT decreased significantly between baseline and year 5 as well as between year 1 and year 5 (**Figure 1B**). This was observed together with a significant decrease of the SRT-DR sub-item of the SRT between baseline and year 1 and between baseline and year 5 (**Figure 1C**). Additionally, a significant increase of both BVMTR sub-items was observed between baseline and year 1 (**Figures 1D,E**).

Raw scores of patients to each cognitive test can be found in **Supplementary Table 1**.

Those results suggest that patients do not display the same learning-effect in verbal memory as it is seen in healthy controls after 5 years of evolution, i.e., patients learn less. Visuo-spatial memory on the other hand, seems to be maintained.

Hippocampal Volume and Connectivity at Baseline, Year 1 and Year 5

Figure 2 shows the evolution of MRI metrics over the three time points in patients. A significant decrease in hippocampal volumes was observed from baseline to year 5 and from year 1 to year 5 (**Figure 2A**). This was present along with a significant increase in

TABLE 1 | Patients demographic, clinical and conventional MRI characteristics.

	Baseline		1 year	5 years
Population	**Patients**	**Controls**	**Patients**	**Patients**
Mean age, years *(SD)*	37.8 (10.4)	40.4 (7.06)	–	–
Sex ratio *(F/M)*	25/7	6/4	–	–
Education level *(high/low)*[a]	20/12	9/10	–	–
Median EDSS score *[range]*[b]	1.5 [0–3]	–	1 [0–3]	1.75 [0–4]$^{\#}$
Median T2 Lesion volume *mL [range]*[b]	0.85 [0.02–25.97]	–	1.56 [0.07–16.65]	2.40 [0.17–20.97]†††
Conversion to MS *n (%)*	28 (87.5)	–	29 (90.6)	29 (90.6)
Normalized brain fraction % (SD)[c]	84.67 (3.43)	85.43 (2.52)	83.95 (3.72)*	84.13 (4.27)
Normalized white matter fraction % (SD)[c]	35.60 (2.83)	37.00 (2.70)	34.44 (3.21)**	34.62 (2.71)$^{\#}$
Normalized gray matter fraction % (SD)[c]	49.07 (2.95)	48.42 (1.79)	49.51 (2.85)	49.51 (3.20)

SD, standard deviation; EDSS, Expanded Disability Status Scale. [a]French baccalaureate/no French baccalaureate; [b]Wilcoxon test; [c]Paired t-test. Comparison between baseline and 1-year follow-up: $^{*}p < 0.05$; $^{**}p < 0.01$. Comparison between 1- and 5-year follow-up: $^{\dagger\dagger\dagger}p < 0.001$. Comparison between baseline and 5-year follow-up: $^{\#}p < 0.05$.

the structural strength of connections between hippocampi and the rest of the brain over the same periods of time (**Figure 2B**). Functional strength, on the other hand, was not significantly altered over time (**Figure 2F**). Additionally, we observed a significant decrease of structural and functional SPL between hippocampi and the rest of the brain when comparing baseline and year 1 and baseline and year 5 (**Figures 2C,G**). As for betweenness centrality and clustering coefficient, they were not significantly altered in structural (**Figures 2D,E**) nor functional (**Figures 2H,I**) hippocampal networks.

As for healthy controls, none of the standardized residuals of hippocampal structural and functional connectivity metrics were significantly altered over time (data not shown).

Link Between Memory Performances and MRI Metrics at Baseline, Year 1 and Year 5

In order to evaluate the associations between patients' scores to memory tests (see **Figure 1**) and MRI metrics (see **Figure 2**) over time, we fitted LME models.

In a first series of models, our dependent variables were *z*-scores of patients to each sub-item of the SRT (LTS, CLTR, and SRT-DR). We found that CLTR *z*-scores were significantly explained by hippocampal volume (Estimate = 0.29; $p < 0.01$; BIC = 238.49); lower scores being associated with lower hippocampal volume. A sensitivity analysis showed that this association was driven by the left hippocampus (Estimate = 0.33; $p < 0.01$; BIC = 236.65). However, there was no contribution of our connectivity metrics to the CLTR. On the other hand, LTS and SRT-DR *z*-scores were not significantly explained by any of the MRI metrics which showed an evolution over time.

In a second serie of models, we explored the sub-items of the BVMTR. BVMTR-DR *z*-scores were significantly explained by hippocampal functional SPL (Estimate = −0.33, $p < 0.01$; BIC = 288.73); better scores being associated with lower values of SPL. A sensitivity analysis showed that this association was driven by the right hippocampus (Estimate = −0.345, $p < 0.001$; BIC = 287.57). There was no contribution of the other MRI metrics to the BVMTR-DR. On the other hand, BVMTR-learning *z*-scores were not significantly explained by any of the MRI metrics which showed an evolution over time.

DISCUSSION

In this study we shed light on the evolution of memory performances throughout time in the context of early MS and observed their association with hippocampal structural and functional alterations. While we confirmed the already reported decrease in hippocampal volume and its association with some memory dysfunction, we provided new data regarding network reorganization compatible with phenomena of compensation. Indeed, we found data interpreted as a progressive increase in connections between both hippocampi and the rest of the brain with preference for reinforcement of short distance connections which were associated with maintained memory performances in some domains.

Hippocampal Volume Loss and Verbal Memory Decline in MS Patients

Hippocampal Atrophy

We observed a significant decrease in hippocampal volume between baseline, year 1 and year 5, indicating progressive tissue alteration from the early stages of the disease. These data are in line with demyelination and neuronal loss that were reported on pathological examinations from post-mortem brain (5). This is also in line with previous *in vivo* MRI studies which have robustly reported hippocampal volume loss in MS patients compared to healthy controls (6, 7); as well as in MS patients across time (9, 26). The differential vulnerability of the hippocampus to MS pathology was confirmed in our data by the observation that no significant evolution was observed across the five-year period in whole brain gray-matter volumes. This stability in whole brain gray-matter volumes over time could be explained by the presence of multiple local atrophies—such as the one reported here in the hippocampus—which are not yet pronounced enough to be visible in the global picture. Fleischer et al. (27) reported a similar result with no significant alteration in GM volume in MS

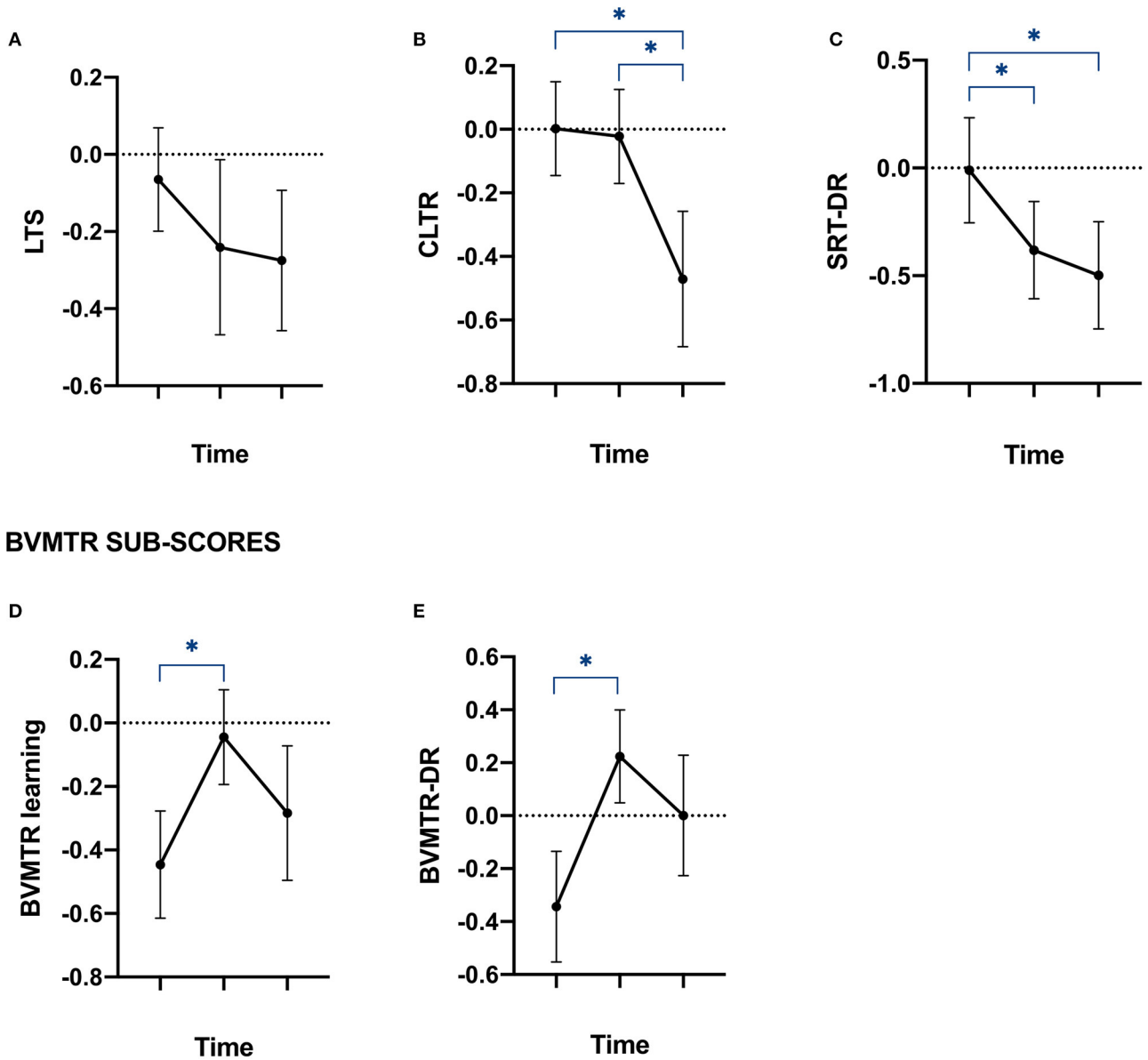

FIGURE 1 | Memory performances of patients at baseline, year 1 and year 5. Plots of patients' *Z*-scores to the tests assessing episodic memory. Data are provided as mean with standard error of the mean. **(A,B)** plots represent the *Z*-scores of patients on each of the Selective Reminding Test (SRT) sub-items, assessing episodic verbal memory performances. **(A)** LTS, long-term storage; **(B)** CLTR, consistent long-term retrieval; and **(C)** SRT-DR = delay recall. **(D,E)** plots represent the *Z*-scores of patients on each of the Brief Visual Memory Test Revised (BVMTR) sub-items, assessing episodic visuospatial memory performances. **(D)** BVMTR = learning; and **(E)** BVMTR-DR = delayed recall. *Correspond to significant *p*-value after Bonferroni's correction for multiple comparison.

patients at different disease stages but a significant reorganization of GM networks.

Relation With Verbal Memory Performances

Regarding the major role of the hippocampus in memory functions (28), the overall decrease in verbal memory performances between baseline, year 1 and year 5 was not unexpected in this context of hippocampal atrophy. Accordingly, our LME models revealed that hippocampal volume was significantly associated with patients' verbal memory performances over time—i.e., it could explain the CLTR sub-item of the SRT. These data confirmed the early memory decline in the context of MS (10, 29) and the implication of specific hippocampal neurodegeneration in such a cognitive decline (9, 26).

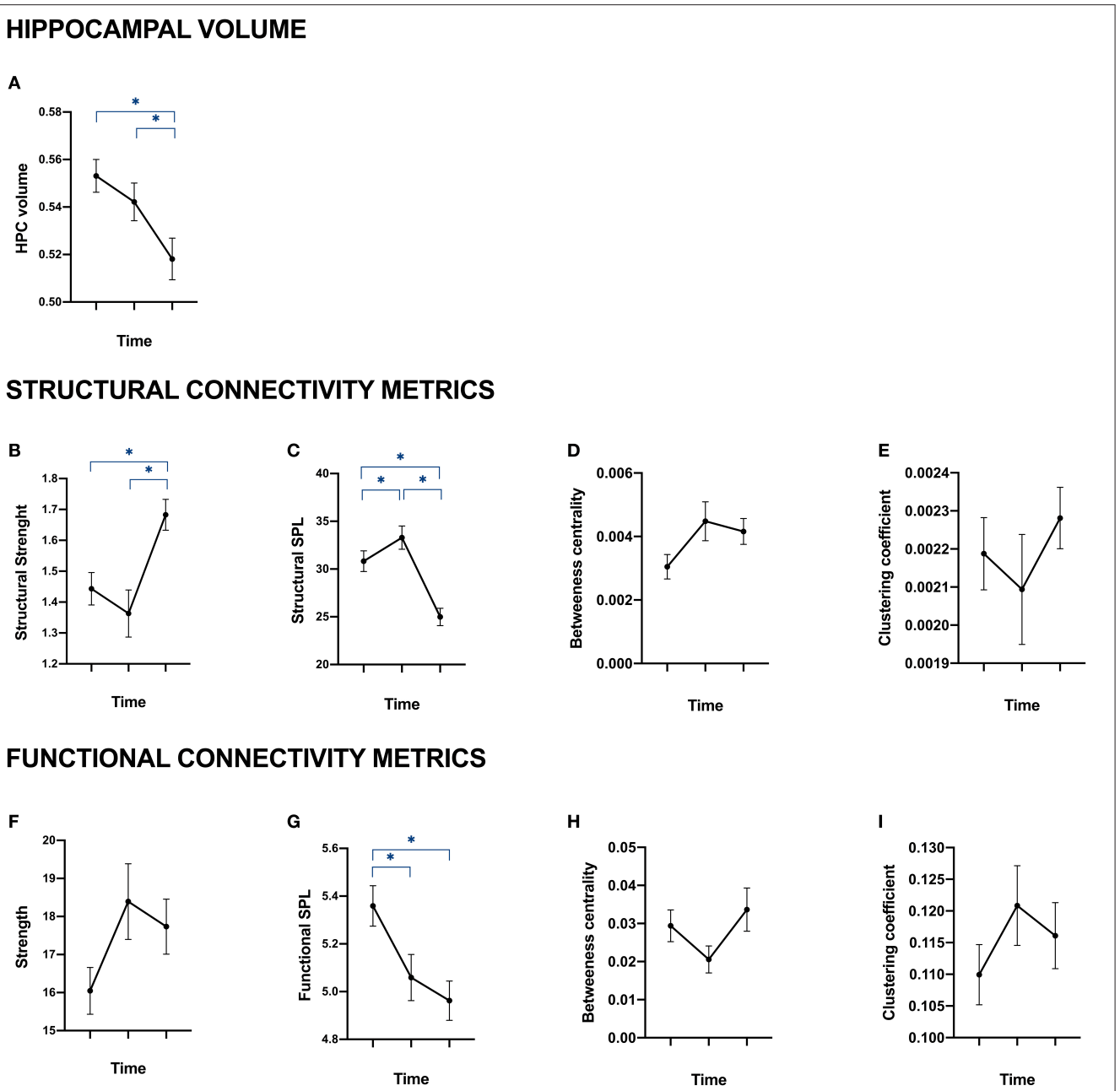

FIGURE 2 | Hippocampal volume, structural and functional connectivity of patients at baseline, year 1 and year 5. Data are provided as mean with standard error of the mean. **(A)** represents the evolution over time of patients' total hippocampal volume (sum of right and left hippocampi). **(B–E)** represent the mean of right and left hippocampi structural connectivity through four metrics coming from graph theory: strength **(B)**, average shortest path length **(C)**, betweenness centrality **(D)** and clustering coefficient. **(F–I)** represent hippocampal functional connectivity through the same metrics: strength **(F)**, average shortest path length **(G)**, betweenness centrality **(H)** and clustering coefficient **(I)**. *Correspond to significant *p*-value after Bonferroni's correction for multiple comparison on age-, sex, education-, and scanner-standardized residuals.

On the other hand, patients' visuospatial memory performances were maintained over time despite the significant hippocampal atrophy that we reported. This raises the possibility that additional mechanisms of compensation could be involved, such as reorganization of hippocampal networks.

Hippocampal Networks Reorganization and Visuo-Spatial Memory Maintenance in MS Patients

As a matter of fact, our analysis revealed an important reorganization both in terms of structural and functional hippocampal connectivity.

Structural Reorganization

First, we observed a significant increase of hippocampal structural strength over time in patients, denoting an increase in the number of connections linking both hippocampi to the rest of the brain. This finding is in line with a previous study reporting greater structural connectivity between both thalami in MS patients compared to healthy controls (30). Such structural plasticity was also reported in many rehabilitation studies of MS patients [see (31) for a review]. Additionally, a significant decrease of hippocampal structural SPL was observed, suggesting a greater efficacy of hippocampal networks, which could be associated to the raise in structural strength that we reported. The increase observed in hippocampal structural strength could be a response to disease pathology as it was previously observed by Fleischer et al. in 2016 (27). They hypothesize that structural reorganization occurs to compensate for ongoing diffuse damage and are essential to maintain network functioning (27). Another interpretation for this increase in detected hippocampal fibers might actually be a reorganization of hippocampal functional connectivity. Indeed, it was previously suggested that activated cells undergo biophysical changes, such as cell swelling and membrane expansion in case of active neuronal firing (32); a phenomenon which could increase the DTI-based detectability of some fibers. Therefore, the increase of hippocampal structural connectivity observed in this study could be considered as a physiological marker of neuronal activation. A qualitative analysis of hippocampal structural connectivity (**Supplementary Figure 1**) indicated an increase in connections between both hippocampi and left temporal regions. Although this was out of the scope of our analysis, future studies should investigate alterations of specific connections and their impact on memory performances.

Functional Reorganization

Indeed, a significant decrease of hippocampal functional SPL was observed, indicating a reinforcement of existing hippocampal functional connections and/or the functional synchronization of the hippocampus with new brain regions. Moreover, a drop in SPL usually characterizes an increase in local, short-distance connections (33). This is in line with a previous study in which it was shown that long-range connections were more severely damaged by multiple sclerosis pathology (34). Fleischer et al. (27) reported similar observations with a strengthening of local connections in the first year after disease onset. Finally, short-distance brain regions are known to be more densely connected both in terms of axonal projections and functional connectivity strength, due to metabolic reasons such as wiring cost (35). The fact that we do not observe a congruent increase in hippocampal functional strength could be explained by an overall equilibrium of functional reorganization. Indeed, even if some hippocampal functional connections are strengthened—mainly short-distance ones—others are weakened with the evolution of the disease (36).

Relation With Visuo-Spatial Memory Performances

LME models revealed that patients' scores to the delayed recall sub-item of the BVMTR were significantly explained by hippocampal functional SPL over time. We also saw that visuo-spatial memory performances—assessed by the BVMTR—were maintained throughout time in MS patients. We can thus speculate that the functional reorganization observed is compensating for hippocampal volume loss, allowing the maintenance of such performances. This hypothesis is in line with Schoonheim et al., suggesting that functional reorganization can act as a compensatory mechanism to attune for structural alterations induced by the disease and mitigate clinical deficits (11). It is also coherent with the results reported by Hulst et al. where the activity of hippocampal memory system was increased in cognitively preserved patients compared to healthy controls when encoding correctly remembered items (13). Additionally, it was previously reported that MS patients' performances in a dual-task were negatively correlated with resting-state networks modularity values; again, suggesting a link between cognitive performances and functional reorganization (37).

However, the BVMTR-learning sub-item of the BVMTR—which did not show impairment across time either—could not be significantly explained by any of our MRI metrics. This gives some perspective on our interpretation of preserved cognitive functions being associated with functional reorganization. Additionally, it is important to notice that even though no significant link was observed between hippocampal structural reorganization and the maintenance of visuo-spatial memory performances, the interplay between both might be of interest. Indeed, it was previously suggested that an increase in structural short-distance connections could be partially compensating for tissue damage (27).

Lateralization of Working Memory Functions

Interestingly, we also saw that the associations discussed above between (1) verbal memory and hippocampal volume and (2) visuo-spatial memory and hippocampal functional SPL, were, respectively, driven by (1) the left and (2) the right hippocampus. This is in line with the commonly accepted idea that verbal working memory is left-lateralized while visual working memory is right lateralized [see (38) for a review].

Healthy Controls Preserved Cognitive Performances and Hippocampal Networks

Healthy controls did not show any significant alterations in memory performances nor in hippocampal MRI metrics over time. This gives us confidence on the robustness of our dataset and allows us to safely interpret alterations seen in patients as consequences of MS.

Strengths and Limitations

Strengths

Strengths of this study include its longitudinal nature over 5 years in a homogeneous population of patients at the early stages of the disease. Additionally, our setting included healthy controls who came back for a 5-year follow-up; a very important advantage since it allows to account for test-retest biases on cognitive tests. Moreover, healthy controls showed no significant evolution in any of the MRI metrics over time, supporting the idea that we can rely on the results observed on our

patient's population and attribute them to the evolution of the disease. Altogether, this study gives strong arguments in favor of functional compensation, with regards to the conflicting results around this question (cf. Introduction).

Limitations

However, there are some methodological limitations to be considered. First, the number of recruited patients followed over the 5 years is limited by missing data which inherently limits the statistical power. In addition, the number of healthy controls who came back longitudinally is low, raising the concern of the robustness of our control group. Nevertheless, as mentioned before, no significant alteration of MRI metrics was observed in controls over time, suggesting that the study design was performant enough to allow stable comparisons. Also, we would like to highlight the fact that it is very rare to have longitudinal data on a control group, especially over a 5-year period, and that still constitutes a great asset of this study.

Second, the ability of tractography algorithms to detect fibers can be affected by the presence of white matter lesions. Nevertheless, a recent study reported that, even though MS lesions impact tractography algorithms, fiber tracking is still possible and anatomically accurate (39). Second, our DTI data were characterized by only 21 non-collinear directions, which could have an effect on our tractography estimations.

In addition, the parcellation used in this study considers the hippocampus as a whole and do not allow the detection of hippocampal sub-fields. This could be a limitation since different memory subtypes might rely on different hippocampal sub-fields. Moreover, we limited our analysis to the hippocampus, while other regions play important roles in verbal and visuospatial memory—such as the right and left medial temporal cortex (40). Lastly, in this study we limited our investigations to four commonly used metrics from graph theory for the evaluation of hippocampal structural and functional connectivity in order to avoid inflation of type I error due to limited sample size; however, other graph measures, such as modularity, could be analyzed in future studies to provide additional insights.

Conclusion

In conclusion, our study demonstrated an important interplay between hippocampal-related structural and functional networks in explaining cognitive performances in the early stages of MS. As the structural damage increases, verbal memory performances decrease while functional reorganization seems to be able to maintain visuo-spatial memory performances with strengthened short-distance connections. Considering those results, a future line of study would be to investigate how such functional reorganization can be stimulated in order to delay the appearance of cognitive impairment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bordeaux University Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AR, MD, BB, TT, and IK: study concept and design. JB, AR, MD, BB, TT, and IK: analysis and interpretation of the data. JB, TT, and IK: statistical analysis and drafting the manuscript. All authors: revised the manuscript for important intellectual content, contributed to the article, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.667531/full#supplementary-material

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Designing a Self-Perception Cognitive Questionnaire for Italian Multiple Sclerosis Patients (Sclerosi Multipla Autovalutazione Cognitiva, SMAC). A Preliminary Exploratory Pilot Study

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Background: Although cognition in multiple sclerosis (MS) is assessed by means of several neuropsychological tests, only a few tools exist to investigate patients' perspectives on cognitive functioning.

Objective: To develop a new questionnaire aimed at exploring patients' self-perception with respect to cognition in Italian MS patients.

Methods: A total of 120 relapsing-remitting MS (RRMS) patients and 120 matched healthy controls (HC) completed a 25-item questionnaire called the Sclerosi Multipla Autovalutazione Cognitiva (SMAC). The Symbol Digit Modalities Test (SDMT), the Delis-Kaplan Executive Function System Sorting Test (D-KEFS ST), the Beck Depression Inventory (BDI-II), and the Fatigue Scale (FSS) were also administered to the patients.

Results: Significantly higher SMAC scores were displayed by RRMS patients compared with HC (30.1 ± 16.9 vs. 23.4 ± 10.4, $p = 0.003$). SMAC inversely correlated with SDMT ($r = -0.31$, $p < 0.001$), D-KEFS ST FSC ($r = -0.21$, $p = 0.017$), D-KEFS ST FSD ($r = -0.22$, $p = 0.015$) and D-KEFS ST SR ($r = -0.19$, $p = 0.035$) and positively correlated with FSS ($r = 0.42$, $p < 0.001$) and BDI-II ($r = 0.59$, $p < 0.001$). Cronbach's alpha coefficient for the questionnaire was 0.94.

Conclusion: Preliminary findings suggest that SMAC is a promising patient-reported outcome to be included in MS neuropsychological evaluation and thus warrants being further tested and developed.

Keywords: multiple sclerosis, cognition, neuropsychology, Patient-Reported Outcomes, self-reports

INTRODUCTION

Several cognitive domains are affected by multiple sclerosis (MS), in particular, sustained attention, information processing speed, memory, and executive functions (1–3). An accurate cognitive assessment has become necessary in the clinical evaluation of MS patients and is currently recognized to have an important prognostic value (4). Indeed, cognition is included in the "No Evidence of Disease Activity" (NEDA) status (5).

Various neuropsychological tests and batteries exist to identify and monitor signs of cognitive impairment in MS, although less literature is available regarding patients' perception of cognitive functioning in daily activities (6), which can be primarily investigated through questionnaires. Specifically, only a few self-reports have been developed for MS, namely, the Multiple Sclerosis Neuropsychological Questionnaire Patient-Form (MSNQ-P) (7) and the Perceived Deficit Questionnaire (PDQ) (8).

The MSNQ-P comprises 15 items focusing on complaints in memory, sustained attention, information processing speed, and behavioral aspects. The PDQ is a self-report questionnaire that consists of 20 items and aims to explore neuropsychological competence in memory, attention, and executive functioning.

Although these questionnaires investigate the main cognitive domains involved in MS, growing research is highlighting further areas of impairment, such as specific aspects of executive functioning (9).

In recent years, both research and clinical practice are increasingly focusing on measures derived directly from patients, the so-called Patient Reported Outcomes (PROs), to understand patients' perception of disease impact and obtain information on quality of life and health status, including cognition (10). Self-reported outcomes, in combination with detailed cognitive testing, may help clinicians to design personalized therapeutic approaches, supporting patient-centered care (11).

Given these premises, purpose of this pilot study is to design a new comprehensive self-report cognitive questionnaire named "Sclerosi Multipla Autovalutazione Cognitiva" (SMAC, "self-perception of cognition in Multiple Sclerosis"), to be used to complete the neuropsychological assessment.

SMAC aims at providing a self-administered measure of perceived cognitive abilities on everyday tasks in MS patients, that can be applied both in clinical and research settings.

METHODS AND MATERIALS

Patients and Controls

In this cross-sectional, single-center study, two cohorts of Relapsing-Remitting MS (RRMS) (12) patients, composed by 30 and 120 patients respectively, were enrolled between May 2018, and October 2019.

The explorative cohort of 30 patients consisted of 24 females and 6 males (F/M = 4). Mean age was 36.0 ± 9.1 years (range 22.0–55.0) and the average education was 14.3 ± 3.4 years (range 8.0–21.0). Thirty age (37.1 ± 10.4 years, range 20.0–55.0), gender (24 females, 6 males, F/M = 4) and education (14.8 ± 2.3 years, range 8.0–20.0) matched subjects participated as Healthy Controls (HC).

The experimental cohort of 120 RRMS patients, was composed by 93 females and 27 males (F/M = 3.4) Mean age was 42.2 ± 10.1 years (range 20.0–60.0) and the average education was 13.5 ± 3.9 years (range 8.0–26.0). Sixty patients were in treatment with oral (40/60) or injectable (20/60) first-line therapies, while the remaining 60 patients were treated with Natalizumab (**Table 1**).

One hundred and twenty age (41.9 ± 13.5 years, range 18.0–65.0), gender (93 females, 27 males, F/M = 3.4) and education (14.3 ± 3.1 years, range 8.0–26.0) matched HC were enrolled in the study (**Table 1**).

Inclusion criteria for RRMS and HC were: (i) age range 18–65 years; (ii) no history/evidence of neurologic or psychiatric disorders (other than MS for patients); (iii) no history of alcohol or drug abuse; and (iv) Italian language as mother tongue.

Each participant gave written informed consent and the study was approved by the Local Ethics Committee.

Methods

SMAC is a self-administered questionnaire designed only in a patient form. Although the informant may be an important source of information, the intent was to focus only on patients' perception of cognitive decline.

The developmental procedure consisted in two phases. In the first phase, a list of 50 items was elaborated and proposed to 30 RRMS patients and 30 HC. In the second phase, 25 items were selected and constitute the final questionnaire that was administered to 120 RRMS patients and 120 HC.

RRMS, not HC, were also assessed by means of the Symbol Digit Modalities Test (SDMT) (13), the Delis-Kaplan Executive Function System Sorting Test (D-KEFS ST) (14), the Fatigue Severity Scale (FSS) (15), and the Beck Depression Inventory II (BDI-II) (16).

Phase 1

The SMAC items were thought to explore the most frequently impaired cognitive functions in MS, thus, clinical experience, literature on neuropsychological impairment and existing self-report questionnaires were considered. A list of 50 items was initially designed grouped into five domains: (1) memory, (2) attention, (3) visuo-spatial abilities, (4) language, and (5) executive functions.

In order to make the questions clearly understandable, many items were supported by examples, consisting of expressions frequently used by Italians to describe recurring cognitive deficits (e.g., "I have the word on the tip of my tongue" or "I know what it is but I cannot recall the name").

Participants were asked to rate each item indicating the frequency of their personal complaints on a four-grade scale, from 0 corresponding to "Never" to 4 corresponding to "Always."

Following the Item Response Theory (17), we then excluded redundant and non-discriminative items or merged some of them. Thus, the initial 50 items were reduced to 25.

TABLE 1 | Demographic and clinical features of the 120 RRMS included in the study and demographic features of the HC.

	RRMS ($n = 120$)	HC ($n = 120$)
Age (years)*	42.2 ± 10.1	41.9 ± 13.5
Education (years)*	13.5 ± 3.9	14.3 ± 3.1
Female/male (ratio)	93/27 (3.4)	93/27 (3.4)
Disease Duration (years)*	11.3 ± 9.5	na
EDSS**	2.0 (1.5–3.0)	na
Disease Modifying Therapies		
First-line		
- Interferon β-1a/b (injective)	6	na
- Glatiramer Acetate (injective)	14	
- Dimethyl Fumarate (oral)	34	
- Teriflunomide (oral)	6	
Second-line		
- Natalizumab	60	
SMAC score*	30.1 ± 16.9***	23.4 ± 10.4

*na, not applicable; SD, standard deviation; IQR, interquartile range. EDSS, expanded disability status scale; SMAC, sclerosi multipla autovalutazione cognitiva. Data are expressed as mean ± SD * or median and IQR **, ***$p < 0.005$.*

Phase 2

The final 25-item SMAC version was proposed to 120 RRMS patients and 120 HC.

For the scoring procedure, patients' answers to the questionnaire were considered as a whole, and therefore scores on the SMAC range from 0 to 100. Subjects were requested to complete the questionnaire referring to the present situation. Time to complete SMAC was about 5 min. Higher scores indicate greater perception of cognitive difficulties. The 120 MS patients further completed the FSS, the BDI-II, the SDMT and the D-KEFS ST. The SMAC, in the Italian and the English translation, is provided in **Supplementary Material**.

Neuropsychological Evaluation: Cognitive Testing

As described above, RRMS were tested with SDMT and D-KEFS ST. SDMT is actually considered the most sensitive test for MS-related cognitive dysfunction and thought to have good reliability and validity (13, 18, 19). This test measures sustained attention, visual tracking, and processing speed. D-KEFS ST was found to be a useful tool to evaluate the executive functions namely categorization abilities, problem-solving skills, abstraction and flexibility of thinking in a sample of RRMS patients (20). This test provides three indexes, namely Free Sorting Categorization (D-KEFS ST FSC), Free Sorting Description (D-KEFS ST FSD) and Sort Recognition (D-KEFS ST SR) (14). The integrity of executive functions was found to associate with a better therapeutic compliance (21).

Self-Report Questionnaires

Fatigue Severity Scale (FSS)

The 9-item FSS was used to measure the impact of fatigue on everyday tasks. Subjects are asked to rate its severity on a 7-point Likert scale. Higher scores indicate greater severity of fatigue symptoms (15).

Beck Depression Inventory II (BDI-II)

The 21-item BDI-II was used to measure depressive symptoms. Items are rated on a 4-point Likert scale. Higher scores indicate more severe depressive symptoms (16).

Statistical Analysis

The sociodemographic differences between the RRMS and HC samples were tested differently according to the data, Student's *t*-test (or the Wilcoxon rank sum test when the normality assumption was not satisfied) for continuous variables and chi-square test categorical variables. Cronbach's alpha was assessed for internal consistency of the outcome of SMAC. In the patient group, correlations between the SMAC and demographics (age, education, and gender), clinical data (disease duration and EDSS), neuropsychological testing (SDMT and D-KEFS ST) and self-report questionnaires (FSS and BDI-II) were explored using Pearson's correlation coefficient. Because of lack of homoscedasticity, the Welch *t*-test was applied to test group differences in SMAC between MS and HC. For within RRMS group comparisons, Student's *t*-test (or the Wilcoxon rank sum test when the normality assumption was not satisfied) was used to explore differences in SMAC between first and second-line therapies and between oral and injectable therapies. All tests were two-tailed and were considered statistically significant when $p < 0.05$. All analyses were conducted in the R programming environment (22).

RESULTS

Demographics and Clinical Features of RRMS and HC

RRMS and HC did not differ in age (W = 7,179, $p = 0.969$), education (W = 8,059, $p = 0.102$) and gender ($p = 1.0$). RRMS patients had a mean disease duration of 11.3 ± 9.5 (range: 0.1–39.0) years and a median EDSS score of 2.0 (interquartile range

TABLE 2 | Correlations between SMAC, demographics, clinical data and neuropsychological testing.

	Mean ± SD	Range	Correlation with SMAC
Age (years)	42.2 ± 10.1	20–60	0.15
Education (years)	13.5 ± 3.9	8–6	−0.010
Disease Duration (years)	11.3 ± 9.5	0–39	0.30**
EDSS (median IQR)	2.0 (1.5–3.0)	0–7	0.06
SMAC (total score)	30.1 ± 16.9	6–86	1
FSS (total score)	3.6 ± 1.7	1–7	0.42**
BDI-II (total score)	9.9 ± 9.3	0–46	0.59**
SDMT (raw score)	53.4 ± 14.6	17–109	−0.31**
D-KEFS ST FSC (raw score)	9.1 ± 3.2	0–16	−0.21*
D-KEFS ST FSD (raw score)	34.8 ± 12.6	0–60	−0.22*
D-KEFS ST SR (raw score)	36.7 ± 13.4	0–60	−0.19*

*$p < 0.05$, **$p < 0.001$. FSS, Fatigue Severity Scale; BDI-II, Beck Depression Inventory-II; SDMT, Symbol Digit Modalities Test; D-KEFS ST FSC, D-KEFS Free Sorting Categorization; D-KEFS ST FSD, D-KEFS Free Sorting Description; D-KEFS ST SR, D-KEFS Sort Recognition.*

(IQR): 1.5–3.0). Demographic and clinical characteristics of the two groups are summarized in **Table 1**.

SMAC in RRMS and HC

Significantly higher SMAC scores were observed in RRMS patients compared to HC (30.1 ± 16.9 vs. 23.4 ± 10.4; W = 5637, $p = 0.003$). A mean score was obtained for each item for both patients and controls. The higher difference in the mean score was found in three items, namely, item 8: "I find it difficult to pay attention for a while (I get easily distracted, I need to take several breaks during extended activities…)" (RRMS vs. HC: 1.61 vs. 0.94); item 24: "I have trouble with simple arithmetical calculations" (1.08 vs. 0.60); and item 5: "I easily forget things I have done recently (books I have read, programs I have watched on TV, conversations I have had with other people…)" (1.37 vs. 0.94).

SMAC Correlated With Disease Duration

SMAC did not correlate with any demographic variables (i.e., age: $r = 0.15$, $p = 0.089$; education $r = -0.10$, $p = 0.266$; gender: $r = 0.17$, $p = 0.054$). Moreover, no correlation was found between SMAC and EDSS ($r = 0.06$, $p = 0.538$), whereas a positive correlation emerged with disease duration ($r = 0.30$, $p < 0.001$). No differences were found in SMAC scores between patients treated with first and second-line drugs (28.8 ± 17.2 and 31.6 ± 16.6, respectively; W = 1,584, $p = 0.257$). In the first line therapy group, no difference was observed between oral and injectable treatments (26.9 ± 16.6 and 32.3 ± 18.3, respectively; W = 484, $p = 0.190$).

SMAC Correlated With SDMT, D-KEFS ST, FSS, and BDI-II

A significant negative correlation was observed between SMAC and SDMT ($r = -0.31$, $p < 0.001$), D-KEFS ST FSC ($r = -0.21$, $p = 0.017$), D-KEFS ST FSD ($r = -0.22$, $p = 0.015$), and D-KEFS ST SR ($r = -0.19$, $p = 0.035$). Positive correlations were observed between SMAC, FSS ($r = 0.42$, $p < 0.001$), and BDI-II ($r = 0.59$, $p < 0.001$) (**Table 2**).

SMAC Internal Consistency

Cronbach's alpha coefficient was 0.94 for the total 25-item scale.

DISCUSSION

The aim of the present study was to design a questionnaire intended to investigate the self-perception of cognitive functioning in Italian MS patients, named "Sclerosi Multipla Autovalutazione Cognitiva" (SMAC). Evidence from literature and professional experience in the field were taken into consideration and carefully discussed.

Compared with the currently existing cognitive self-reports in MS, SMAC considers a greater range of cognitive domains, including language abilities and a wider spectrum of executive functions, both objects of growing interest in MS (23, 24). Various scenarios are presented, most of them providing examples that address everyday life situations, such as forgetting appointments, having a word on the tip of the tongue, losing one's train of thought, and so on. The questionnaire proved to be easy to administer and relatively short-lasting; indeed, the 25 items can be answered in about 5 min and are easily scored. It disclosed a high internal consistency.

RRMS reported higher perception of cognitive difficulties than HC. The main differences in mean item scores between the two groups were observed for items regarding sustained attention, short-term memory, slowing information processing speed, and working memory. These domains constitute the core of cognitive decline in MS patients (3). In line with previous research (7, 25) self-perception of cognitive deficits was not associated with demographic variables such as age, education, or gender. However, disease duration positively correlated with SMAC, indicating that while the disease progresses, the perception of cognitive functioning increases.

Regarding neuropsychological testing, patients' total scores on the SMAC showed small negative correlations with both SDMT and D-KEFS ST suggesting that a worse perception of cognitive functioning is associated with drops in performances of attention, information processing speed, and flexibility of thinking. Higher

positive correlations were observed between SMAC and self-assessed fatigue and depression. These preliminary findings are consistent with results from previous studies using similar tools (25–30), which reported that questionnaires completed by patients showed absent or low correlations with outcomes of neuropsychological assessment while associations with measures of fatigue and depression were higher.

These findings could indicate that clinical care would benefit from including self-reported metrics of cognition in MS neuropsychological assessments. While neuropsychological testing is needed to identify impairment in specific cognitive domains, self-report questionnaires might help clinicians to collect information about how those difficulties are perceived by patients.

Finally, no difference was found in SMAC scores between patients treated with first- and second-line treatments, nor between patients treated with oral or injectable drugs. This suggests that SMAC was not influenced by pharmacological interventions.

This preliminary work presents some limitations. Since only RRMS patients were enrolled, future studies would benefit from testing different clinical phenotypes, especially patients with very short disease duration. Although the number of enrolled patients and HC was tailored on the study aims, SMAC needs to be tested and possibly validated in a larger number of patients. Finally, since neuropsychological assessment did not cover all the cognitive domains, further research has to include more comprehensive cognitive testing.

CONCLUSION

The neuropsychological evaluation of MS patients is increasingly taking into consideration so-called Patient-Reported Outcomes (31–33), thus recognizing the relevant role of patients' perception of disease severity and treatment efficacy as a fundamental aspect of patient's management. Indeed, self-reports are clinically important since they contribute to better depict patients' profiles, thus supporting clinicians in the recognition of the early signals of disease progression and guiding toward neuropsychological rehabilitation, psychotherapy, or other pharmacological interventions.

Although further research is needed, the preliminary findings of this pilot study suggest that SMAC deserves to be further tested as a reliable and easy-to-perform questionnaire to investigate the self-perception of cognition in MS, with application in both clinical practice and research.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Local Ethic Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AR and FO designed the study and collected neuropsychological data. MP and PG equally contributed to the final version of the manuscript. SZ, SMi, PP, and FR collected clinical data. IM and EC collected neuropsychological data. MN and MM review the paper and performed data analysis. SMo designed the study and review the paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.668933/full#supplementary-material

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REVIEW
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Structural and Functional Connectivity Substrates of Cognitive Impairment in Multiple Sclerosis

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Cognitive impairment (CI) occurs in 43 to 70% of multiple sclerosis (MS) patients at both early and later disease stages. Cognitive domains typically involved in MS include attention, information processing speed, memory, and executive control. The growing use of advanced magnetic resonance imaging (MRI) techniques is furthering our understanding on the altered structural connectivity (SC) and functional connectivity (FC) substrates of CI in MS. Regarding SC, different diffusion tensor imaging (DTI) measures (e.g., fractional anisotropy, diffusivities) along tractography-derived white matter (WM) tracts showed relevance toward CI. Novel diffusion MRI techniques, including diffusion kurtosis imaging, diffusion spectrum imaging, high angular resolution diffusion imaging, and neurite orientation dispersion and density imaging, showed more pathological specificity compared to the traditional DTI but require longer scan time and mathematical complexities for their interpretation. As for FC, task-based functional MRI (fMRI) has been traditionally used in MS to brain mapping the neural activity during various cognitive tasks. Analysis methods of resting fMRI (seed-based, independent component analysis, graph analysis) have been applied to uncover the functional substrates of CI in MS by revealing adaptive or maladaptive mechanisms of functional reorganization. The relevance for CI in MS of SC–FC relationships, reflecting common pathogenic mechanisms in WM and gray matter, has been recently explored by novel MRI analysis methods. This review summarizes recent advances on MRI techniques of SC and FC and their potential to provide a deeper understanding of the pathological substrates of CI in MS.

Keywords: multiple sclerosis, structural connectivity, functional connectivity, cognitive impairment, substrates

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INTRODUCTION

It has been nearly 150 years since Charcot described cognitive impairment (CI) in multiple sclerosis (MS) patients as "enfeeblement of memory" and "concepts formed slowly" (1). The importance of CI in MS was reinforced a few decades ago, after a long period of underestimation (2). CI in MS patients can affect multiple domains including attention, information processing speed (IPS), memory, and executive control (3, 4) and may be present since the early disease stages, being more prevalent in the progressive forms (5) (see **Box 1** for a definition of MS phenotypes). Recently, in order to overcome the heterogeneity of CI in MS, some studies have proposed cognitive phenotypes, characterized by the prevalent impairment of a specific cognitive domain, based on predefined

BOX 1 | MS phenotypes

Clinically isolated syndrome (CIS)
A monophasic clinical episode with patient-reported symptoms and objective findings reflecting a focal or multifocal inflammatory demyelinating event in the central nervous system, developing acutely or subacutely, with a duration of at least 24 h, with or without recovery, and in the absence of fever or infection, similar to a typical MS relapse (attack and exacerbation) but in a patient not known to have MS (14).
Relapsing–remitting MS (RRMS)
Presence of relapses with stable neurological disability in between them (14).
Secondary progressive MS (SPMS)
Progressive course following an initial relapsing–remitting course (14).
Primary progressive MS (PPMS)
Progressive course from disease onset (14).

cutoff values (6, 7) or latent profile analysis (8). Furthermore, the involvement of cognitive reserve has been suggested to partly explain the "clinicoradiological paradox" in MS patients without CI despite the evidence of brain damage (9–13).

Magnetic resonance imaging (MRI) may contribute to improve the current partial understanding of the pathogenic mechanisms of CI in MS. Over the last decade, several MRI measures have been proposed as biomarkers of CI in MS, including white matter (WM) lesion load and distribution, gray matter (GM) lesions, and cortical and deep GM atrophy (9, 15).

However, abnormalities in MS are not simply confined to a single brain region but rather tend to spread via axonal pathways, thus involving other regions (16). More recently, taking into account the complex topological organization of the human brain, advanced MRI techniques assessing structural connectivity (SC) or functional connectivity (FC) have been developed and applied to various neurological conditions, including MS (17).

The aim of this review was to summarize the recent applications in MS of MRI-based SC and FC approaches to the assessment of the pathogenic substrates of CI in different cognitive domains, starting with a brief methodological description. Finally, future directions and challenges will be discussed.

For all these purposes, this review included scientific literature of the last 10 years from PubMed using the search terms "cognition," "cognitive impairment," "cognitive deficits," "cognitive decline," "cognitive dysfunction," "multiple sclerosis," "neuropsychological evaluation," "connectivity," "functional connectivity," "structural connectivity," "network," "cognitive phenotypes," "cognitive reserve," "fMRI," "resting-state fMRI," "diffusion MRI," "diffusion tensor imaging," "tractography."

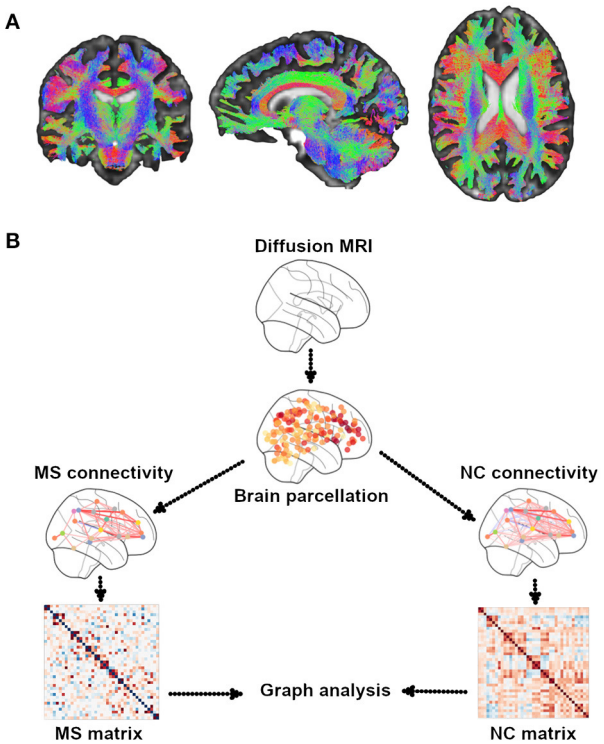

FIGURE 1 | Illustrative example of WM tractography. **(A)** Different colors show the three systems of WM tracts: red for commissural (laterolateral direction), green for association (anterior–posterior direction), and blue for projection (superior-to-inferior direction). WM tractography were overlaid onto MNI standard brain. **(B)** A general overview of the pipeline of graph theory analysis for the assessment of structural brain networks. A network or a graph is a collection of vertices (nodes) and corresponding pairwise connections (edges). A comprehensive set of all pairwise connections in the brain defines the topology of a brain network, providing a complete connectivity diagram of all connections among nodes and edges, that is, a connectome. There are four essential steps in performing a graph theory analysis: (1) defining nodes: nodes are brain regions of interest (ROIs), typically derived from an anatomical parcellation of an imaging dataset; (2) defining edges: edges reflect the relationship between each node pair; they can be streamline connections derived from DTI tractography; (3) constructing a network: this step integrates all the information from nodes and edges in order to generate a complete connectivity map; the simplest representation of a network is using a two-dimensional matrix (i.e., a connectivity matrix); (4) graph theory analysis: currently, the most commonly used method to assess the characteristics of a network; it provides various measures of network topology. MS, multiple sclerosis; NC, normal controls.

ASSESSMENT OF BRAIN CONNECTIVITY

Measuring SC

Diffusion MRI is a type of sequence that is sensitive to the random microscopic motion of water molecules (18), thus providing information on the microstructure of WM fiber tracts noninvasively. Since the introduction of diffusion tensor imaging (DTI) (19), which assumes a Gaussian diffusion of water molecules, images, and corresponding indices derived from the tensor model, such as fractional anisotropy (FA) and mean, axial, and radial diffusivities (20), were used to assess structural integrity along tractography-derived WM fiber tracts, a proxy for SC (18, 21, 22) (**Figure 1**). Because of the limitations of traditional DTI regarding regions with crossing fibers and multiple fiber orientations within a single voxel, alternative diffusion methods have been proposed. They include diffusion kurtosis imaging (DKI) (23), diffusion spectrum imaging (DSI) (24), high angular resolution diffusion imaging (HARDI) (25), and neurite orientation

dispersion and density imaging (NODDI) (26), which assess, respectively, the non-Gaussian behavior of water diffusion, the likelihood of water diffusion along any space direction, the orientation density function using less sampling intensive spherical q-space acquisitions, and the angular variation of neurite orientation. These methods offer the potential added value of a higher sensitivity to pathological changes over traditional DTI (27, 28). However, long scan time and mathematical complexities have thus far hindered their use in the clinical setting.

BOX 2 | Summary of different approaches assessing brain connectivity

Structural connectivity

Graph theory methods

The connectivity matrix, a squared N × N matrix representing connectivity between nodes, is typically constructed from a combination of brain tractography and any type of parcellation (21):

Anatomical parcellation: Node definitions based on *a priori* anatomical information, such as sulci and gyri, or anatomically predefined ROI (34)
Strength: Rapid and intuitive parcellation
Limitations: Low resolution, large variations in node size

Random parcellation: Brain is randomly parcellated into discrete nodes of similar size (34)
Strength: Minimizes node size variations
Limitations: Unclear validity/reliability

Functional parcellation: Node definitions based on *a priori* functional information, such as coordinates of peak activations or meta-analytic results (34)
Strength: Hypothesis-driven, equal node size
Limitations: Definitions are data-specific, may miss some regions, difficult to apply to diffusion MRI data

Voxel-based parcellation: Each image voxel represents a distinct node (34)
Strength: Data-driven, high resolution
Limitations: Computationally intensive

Data-driven methods

Model-free; connectivity is identified by the multivariate methods:

Independent component analysis: Performs a linear decomposition on the whole brain tractography matrix for identifying structural connectivity (32)
Strength: Data-driven
Limitations: The estimated independent components and the respective mixing matrix can contain both positive and negative values, leading to challenges in the interpretation of negative weights.

Nonnegative matrix factorization: An unsupervised technique for extracting connectivity components from diffusion MRI data, both at the group and individual level (33)
Strength: Data-driven, easy for interpretation
Limitations: Biased decomposition, computationally intensive

Functional connectivity

Statistical dependency (i.e., Pearson correlation coefficient) between signals measured from different "brain units" is thought to be indicative of FC (35), based on:

- Task fMRI (36)
  Strength: Directly reveals differences related to a task (e.g., cognitive, motor)
  Limitations: Patients may have difficulty in completing the scan, interpretation of fMRI results during cognitive tasks can be difficult when task performance differs across patients
- Resting fMRI (35)
  Strength: Easier for patients to complete the scan
  Limitations: It may provide just a partial picture of the brain's functional architecture, missing the functional reorganization shown by task fMRI

Static edge-based functional connectivity: Edge-based summary measures include full or partial correlation and mutual information (35)
Strength: Easy to implement
Limitations: Cannot provide direction information of FC, interpretation challenges in case of brain pathology

Effective connectivity: Evaluates the directionality and strength of FC between pairs of "brain units" (35)
Strength: It can provide direction information of connectivity
Limitations: Difficult to find an appropriate model for fast changes in effective connectivity

Dynamic functional connectivity: Reflects variations in FC over time (35)
Strength: Captures time-varying FC
Limitations: Signal-to-noise ratio of MRI data may be a practical limitation for FC assessment

Two main approaches of tractography exist, referred to as deterministic and probabilistic (21). The former reconstructs WM fibers assuming a single orientation within each voxel, whereas the latter assumes an orientation distribution of such fibers (21). SC across the brain is typically built up by first defining a pair of parcellated regions (see **Box 2** for the parcellation details) and then running tractography and finally assessing connectivity measures from the connecting WM streamlines (21). Each region is defined as a "node," whereas WM connections are considered as "edges" of the structural network (27, 28). Within this framework, graph analysis can be performed on the SC matrix and allows deriving various network measures of integration (path length, global efficiency), segregation (clustering coefficient, transitivity, local efficiency, modularity), centrality, motifs, resilience (degree, assortativity coefficient), and other features (small worldness, rich club coefficient) (28, 29) (see **Box 3** for details on graph theory measures). These measures help unveil the topological features

BOX 3 | Graph analysis glossary in the review

Node
Neurons and/or brain regions (37)
Edge
Functional (29) or structural (38) relationships between brain regions
Nodal strength
Sum of the weighs across all connections associated with that node (39)
Path length and efficiency
Path length is the minimum number of edges that must be traversed to go from one node to another (28).
The average inverse shortest path length is a related measure known as the *global efficiency* (29).
Path length and global efficiency measure the ability of parallel information exchange across the whole network (40).
The *local efficiency* of a particular node is the inverse of the average shortest path connecting all neighbors of that node, measuring the information transfer in the immediate neighborhood of each node (41).
Clustering coefficient and transitivity
The fraction of triangles around an individual node is known as the *clustering coefficient*, and is equivalent to the fraction of the node's neighbors that are also neighbors of each other.
Clustering coefficient reflects the network segregation (29), the ability for specialized processing to occur within interconnected groups of brain regions (41).
The *transitivity* is the ratio of triangles to triplets in the network and is an alternative to the clustering coefficient (29).
Modularity
It measures the quality of division of a network into modules (41).
Centrality
It measures the relative importance of a node or edge within the overall architecture of a network (37).
Motif
Small (e.g., three or four nodes) patterns of local connectivity that occur in the network with a statistically surprising frequency (29)
Degree
Number of edges attached to a given node (37)
Hub
A node occupying a central position in the overall organization of a network (37)
Rich club
A set of high-degree nodes in a network to be more densely interconnected than expected on the basis of their node degree alone (37). The *rich club* effect of brain networks plays an important role in the information transmission across the brain (41, 42)
Feeder
Connections linking rich club nodes to nonrich club nodes (43).
Assortativity and hierarchy
Assortativity is a measure of the tendency for nodes to be connected to other nodes of the same or similar degree (28).
Hierarchy is the tendency of hubs to connect to nodes that are not otherwise connected to each other (44).
Increased assortativity and reduced hierarchy indicate an impaired wiring efficiency at a system level (44).
Mean network degree
The average degree of all network nodes and a measure of network density (29)
Module efficiency
Evaluating the communication efficiency both within and between structural networks (45). Intramodule efficiency: measures the global efficiency of the parallel information transfer within the module; intermodule efficiency: measures the global efficiency of the parallel information transfer between two different modules (45).
Module: a group of nodes that maintains a large number of mutual connections and a small number of connections to nodes outside their group (37)
Small worldness
A network that shows a level of clustering higher than that observed in random networks and an average shortest path length that is equal to that observed in random networks (37)
Network efficiency
Assessment of the exchanging information performance of small-world brain functional networks (40)
Communicability
Measure of network integration. It accounts for the contribution of all possible walks between a pair of nodes, reflecting a network's capacity for parallel information transfer under a diffusion model of information flow (46). Walk: a path in a network that is allowed to visit the same nodes and edges on multiple occasions (46)

of brain structural networks and can be used to study the relationship with cognitive functions (30). In contrast to graph analysis, data-driven mapping approaches such as independent component analysis (ICA), a multivariate method identifying single brain structural networks (31, 32), and nonnegative matrix factorization (NMF), an unsupervised technique based on structural network parcellations from DTI data (33), may be used, by providing a different way of assessing disrupted SC in pathological conditions (31, 32) (see **Box 2** for strengths and limitations of SC assessment).

Measuring FC

Functional MRI (fMRI) is a well-established method able to detect at the level of GM regions changes in the blood oxygenation level–dependent (BOLD) signal, which indirectly reflects neuronal activation *in vivo* (47). Two fMRI paradigms exist: the first is task based, assessing the brain regions activated during a specific task (e.g., cognitive, motor) (48), whereas the second is resting-state fMRI, measuring the similar spontaneous fluctuations of the BOLD signal between brain regions—FC—reflecting "intrinsic" functional relationships (35).

A "brain unit" can be viewed as a spatially defined functional processing unit at different levels, including parcellated brain regions, regions of interest (ROIs), or resting-state networks (**Figure 2**) (35, 49, 50). In this context, FC can be considered in terms of statistical similarity (i.e., full or partial correlation, mutual information) between signals measured from pairs of brain units (35). For instance, after defining an ROI as "seed," a correlation map with another ROI or the whole brain can be estimated (35). Moreover, FC can be assessed, similarly to SC, in the graph theory framework, using the measures listed in the previous paragraph. Finally, FC may be derived at voxel level, using dual regression on the ICA decomposition maps of resting fMRI (51).

Context-dependent connectivity between brain units during a task fMRI (49) and intrinsic connectivity between time series of brain units during resting fMRI (35) can also be obtained. In addition to the traditional "static" connectivity, variations in the FC over time—dynamic FC—and in the directionality and strength of FC between pairs of brain units—effective connectivity—can be assessed (35, 52) (see **Box 2** for the strengths and limitations of FC assessment).

In order to investigate the substrate of CI in MS, FC may be used directly in the statistical models or fed into the graph theory framework to extract corresponding measures (30, 53).

CONNECTIVITY SUBSTRATES OF CI IN MS

Global Cognition

Various tools are available to explore cognition in MS (4, 54), from short screening tests to full neuropsychological batteries covering a wide range of cognitive performances (54–56). The former includes the Brief International Cognitive Assessment for Multiple Sclerosis (57) and the Multiple Sclerosis Outcomes Assessment Consortium (58), whereas the latter comprises the Brief Repeatable Battery of Neuropsychological tests (59) and the Minimal Assessment of Cognitive Function in MS (56). Global

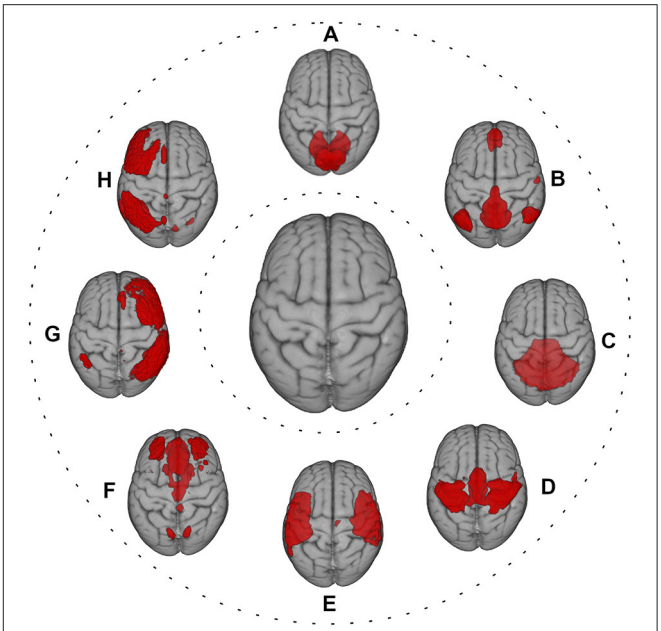

FIGURE 2 | Red color shows the most representative resting-state networks, reflecting large-scale functional patterns, overlaid onto MNI standard brain. **(A)** visual network; **(B)** default mode network; **(C)** cerebellum network; **(D)** sensorimotor network; **(E)** auditory network; **(F)** executive control network; **(G)** right frontoparietal network; **(H)** left frontoparietal network.

CI in MS can be defined in different ways: (i) performance $\leq$1.5 to 2 standard deviations (SDs) from the mean normative values in 20 to 30% of tests, (ii) impairment $\geq$1.5 to 2 SDs in at least two cognitive domains, (iii) use of composite scores, (iv) a combination of the above systems (60).

Structural Connectivity

Relapsing–remitting (RR) and secondary progressive (SP) MS patients with CI both showed a widespread reduction in two key measures of SC, such as local efficiency and nodal strength, suggesting the presence of a network collapse or its inability to compensate for such impairment (61). It is thought that CI in MS may be the result of a "disconnection syndrome" (17, 62). Such hypothesis was investigated in RRMS patients at whole-brain level in terms of path length, and it was found that impaired long-range rather than short-range FA-based connections had stronger correlation with decreased structural network efficiency, as well as with worse global CI measured by a composite score. These findings suggest that MS pathology mainly interrupts structural pathways connecting remote brain regions playing an important role for global cognition (63).

Functional Connectivity

Disruption of global FC, as shown by both reduction in mean network degree, global efficiency and hierarchy, and increase in path length and assortativity, contributed to distinguish MS patients with CI [benign MS (BMS), RR, SP] from those without CI and healthy controls (64).

In pediatric RRMS, patients with preserved cognition showed, compared to healthy controls and patients with CI, an increased FC in the left frontoparietal network, indicating that FC may partially contribute to compensate for disease-related structural damage and that it may gradually fail over time with the accrual of such damage (65). In adult RRMS, increased FC in bilateral frontoparietal networks was found in patients with preserved cognition, compared to healthy controls and patients with CI (66). On the other hand, in a large study including RR, SP, and primary progressive MS (PPMS), increased FC between thalamic and temporal regions (i.e., hippocampus, parahippocampal gyrus, superior temporal cortex) was found in patients with CI, compared to patients without CI, probably reflecting maladaptive mechanisms toward cognition (67). Moreover, default mode and frontoparietal networks showed increased FC with the rest of the brain in an MS population with CI including different phenotypes (RR, SP, and PP), suggesting that CI in MS may be due to abnormal communication of hub-rich networks (68). Decreased FC in the dorsal attention and default mode networks were also identified in adult RRMS patients with CI, probably reflecting a failure of compensatory mechanisms (66). In PPMS patients with CI, widespread seed-based functional network reorganization was found. In particular, there was decreased FC of the dorsal attention network with the insula and occipital cortex compared to PPMS patients without CI, whereas decreased FC of the executive control network with the insula and right frontoparietal network as well as between the dorsal attention network and the right frontoparietal network was observed compared to healthy controls (69).

SC–FC Coupling

In patients with clinically isolated syndrome (CIS), a stronger structural–functional coupling, reflected by the higher correlation coefficient between structural and functional networks (70), was able to predict worse global CI (70). This suggests that brain ability in reorganizing functional networks may diminish at later disease stages so that it can no longer compensate for MS-related structural damage (70).

Main Findings

- Decreased structural and functional network integration
- Increased structural and functional network segregation
- Altered FC in the default mode, dorsal attention, and frontoparietal networks.

Attention

Approximately 10% of MS patients experience attention impairment (15), which could be evaluated by either Symbol Digit Modalities Test (SDMT) (71) or Paced Auditory Serial Addition Test (PASAT) (71). Basic attention tasks (i.e., repeating digits) are mostly unaffected in MS patients (3). Impairments are more common in sustained and divided attention, where patients are asked to attend several tasks simultaneously (3).

Structural Connectivity

Globally, in RRMS, lower PASAT correlated with measures of SC disruption such as reduced global and local efficiency and clustering coefficient (72). Meanwhile, reduced efficiency showed a close correlation with larger WM lesion volume (LV), underlying the role of lesions as a contributor to structural network disruption in RRMS (72). Another study showed that reduced global efficiency in SC may help explain decreased SDMT across different MS phenotypes (RR, SP, PP) (73).

Locally, in RR and SPMS, decreased nodal strength in the frontoparietal network, mainly driven by WM LV, correlated with worse PASAT, underlying the importance of the SC within such bilateral network (74).

Functional Connectivity

A reduction in the whole-brain static interhemispheric FC was able to explain well in RRMS worse attention, as measured by decreased SDMT and PASAT performances (75). In addition, better PASAT performance was associated with weaker whole-brain dynamic interhemispheric FC, suggesting that preserved attention in RRMS may be mediated by a smaller flexibility in such a type of connectivity (75).

Regionally, decreased FC in the dorsal attention and visual networks was shown in RRMS patients during a visual attention task (76). On the other hand, increased FC in the frontoparietal network, a hub-rich network, with the rest of the brain (both peripheral and nonhub regions) correlated with worse attention in an MS population including RR and progressive forms (68). Moreover, results of an interventional study showed in RRMS patients that, after 12-week computer-assisted rehabilitation of attention, FC within executive control, salience, and default mode networks increased and correlated with improved attention (77).

Main Findings

- Decreased structural network integration and segregation
- Altered SC and FC in the frontoparietal network.

Information Processing Speed

IPS represents the amount of work performed within a time limit (e.g., number of items completed) (54) and is often assessed in MS by SDMT (71) or PASAT (71). IPS is the most commonly affected cognitive domain in all MS phenotypes (3), with a prevalence of 27 to 51% (15).

Structural Connectivity

At whole-brain level, it was shown in RRMS patients a reduced strength in rich-club and feeder (i.e., between hub and nonhub region) connections, reflecting widespread structural disconnection across the brain and a correlation of it with reduced IPS as measured by PASAT (78). In patients with CIS, increased structural clustering coefficient, reflecting the strengthening of short-distance connections preserving local information flow, correlated with worse IPS, as measured by a computerized speed cognitive test, a novel test for IPS (79). In RRMS, decreased efficiency (both global and local) and clustering coefficient across the brain correlated with lower PASAT (72). Moreover, in a heterogeneous MS population (RR, SP, PP), decreased global efficiency across the brain correlated with worse SDMT (73). Based on NODDI data, CIS patients showed that higher whole-brain modularity coefficient was associated with worse IPS as measured by SDMT (80). Of note, the standardized regression coefficient describing such relationship was greater when the modularity coefficient was obtained with NODDI data

than with conventional DTI, indicating a better sensitivity of NODDI for MS (80).

Beyond whole-brain alterations, SC disruption in various structural networks showed cognitive relevance in MS. Module efficiency, which evaluates the communication efficiency both within and between structural networks, was found decreased in RRMS patients within visual network, between visual and deep GM networks, and between default mode and frontoparietal networks (45), and such reductions were correlated with lower PASAT (45). In another study, a close correlation between lower SDMT and reduced global efficiency in the default mode network was found in RRMS patients with and without CI, although the decrease in such network measure was more pronounced in the former group (81).

Functional Connectivity

Only one study assessed the relevance for IPS of the whole-brain FC, whose increase correlated with decreased IPS, as measured by SDMT, in a large and heterogeneous MS population (RR, SP, PP) (82).

The relevance for IPS of the frontoparietal and default mode networks was found not only for SC, as mentioned previously, but also for FC. Indeed, it was shown in an MS population with CI including different phenotypes (RR, SP, PP) that increased FC of these two networks with the rest of the brain correlated with worse IPS (68). During intrascanner SDMT, RR and SPMS patients with IPS impairment showed, compared to healthy subjects, an opposite direction of the effective connectivity in the frontoparietal networks (83). Specifically, the FC direction in such networks was from right dorsolateral prefrontal to right supplementary motor cortex and from right inferior parietal to left superior parietal cortex (83). In addition, in RRMS patients, a higher FC within the default mode network, specifically between medial prefrontal and frontal pole regions, appeared to facilitate performance stability during a computerized IPS test (84). The role of the default mode network in preserving IPS in RRMS patients was confirmed by the correlation between a larger increase in dynamic FC within such network from resting- to task-state and a better performance of intrascanner SDMT (85). Another study found that increased FC in the left frontoparietal network correlated with better IPS in both RR and SPMS patients (86). Moreover, an increased FC within the salience network, also involved in effective IPS, was found in RR and SPMS (86). Of note, in the same study, only in RRMS patients an FC increase within default mode network showed correlation with worse IPS (86).

In another study, worse IPS correlated with increased FC both within deep GM and between deep GM and cortex in advanced RRMS, and such a correlation further increased in SPMS (87).

After 8-week computer-aided cognitive rehabilitation, RRMS patients with CI showed IPS improvement in parallel to an increase in the default mode network FC at the level of the posterior cingulate and bilateral inferior parietal cortices (88).

Main Findings

- Decreased structural network integration.
- Increased structural network segregation.
- Altered SC and FC of the frontoparietal and default mode networks.

Executive Control

Executive control refers to the cognitive ability needed for complex goal-directed behavior and adaptation to environmental changes or demands, including planning, anticipating outcomes, and appropriately directing resources (3). This cognitive domain can be evaluated by Delis–Kaplan Executive Function System Sorting test (89), Stroop word–color test (ST) (90), and Controlled Oral Word Association Test (91). A 15 to 28% of MS patients usually experience deficits in the executive control domain (15).

Structural Connectivity

Worse executive control in RRMS patients correlated with decreased structural nodal strength in the frontoparietal networks, deep GM structures and insula (74), and within sensorimotor, dorsal attention, left frontoparietal, and default mode networks (92). In another study on SPMS patients where structural networks were obtained using ICA, the component including disrupted supratentorial WM projection tracts and limbic association tracts showed correlation with worse executive control (93).

Functional Connectivity

At whole-brain level, better executive control correlated with both higher dynamics and stronger stationary FC in RRMS (94).

Alterations of regional FC also showed relevance for executive control in MS. Indeed, the presence of "extra effective" (i.e., absent in the FC pattern of healthy subjects) connections during ST resulted different across MS phenotypes (95). In particular, worse executive control correlated with lower FC from left posterior parietal to dorsal anterior cingulate in BMS and with higher FC from right to left insula in SPMS, whereas no correlation was found in RRMS. These findings may reflect the fact that these three MS phenotypes tend to use distinctive mechanisms during a demanding executive control task (95). Another study demonstrated that in RRMS patients with executive control impairment, improvement after computer-assisted cognitive rehabilitation was associated with increased FC between anterior cingulate and frontoparietal cortices of the corresponding network (96). In presence of worse executive control performance during ST, PPMS patients showed, compared to healthy subjects, reduced effective connectivity from left ventromedial prefrontal cortex and increased effective connectivity from left dorsolateral prefrontal cortex to regions of the right frontoparietal network (97), all these abnormalities having a probable maladaptive meaning.

Main Findings

Altered SC and FC of the frontoparietal networks.

Working Memory

Working memory refers to the cognitive system that retains information in mind while performing complex tasks such as reasoning, comprehension, and learning (98). Working memory can be measured by various cognitive tests such as PASAT (9,

54), Letter–Number Sequencing, and Spatial Span subtests, and can be divided into two processing levels, namely, maintenance and manipulation (99). Impairment in working memory has been detected since the early MS stage (100) and across disease phenotypes (101). A 27 to 44% of MS patients showed a decline in working memory over time (3).

Structural Connectivity

An important role for working memory in MS was demonstrated by structural integrity of the frontoparietal network. Decreased FA along the left superior longitudinal fascicle, which is one of the major WM tracts in the left frontoparietal network, correlated with lower working memory in RRMS, because of the disruption of the connections to the prefrontal regions implicated in this cognitive domain (102). As an extension, RR and SPMS patients showing decreased global and local efficiency in the frontoparietal network also showed worse working memory (103). In addition, a study on ICA-based structural networks in SPMS suggested that microstructural damage, assessed by reduced FA, along the supratentorial WM projection and limbic association tracts may contribute to the working memory deficit (93).

Functional Connectivity

Patients with early MS (i.e., CIS and RRMS) showed increased whole-brain functional network modularity (i.e., diminished functional integration between separate functional modules), and this correlated with worse working memory (104). In RRMS patients, better working memory, as measured by PASAT, was associated with smaller flexibility (i.e., more stability) of the interhemispheric dynamic FC involving temporal regions, anterior cingulate gyrus, and parietal regions (75).

Two studies assessed the improvement in working memory performance after a targeted computerized cognitive training. In the first one, it was found in a small group of patients with juvenile MS a less decrease (i.e., a relative increase) in FC between the subcomponents of the default mode network, probably reflecting training-induced plasticity (105). In the second one, performed in adult RRMS patients, it was shown that increased FC between anterior cingulate cortex and right middle frontal gyrus correlated with better executive control, whereas between anterior cingulate cortex and right inferior parietal lobule correlated with better processing speed, with both mechanisms contributing to the improvement in working memory (96). After receiving high-frequency repetitive transcranial magnetic stimulation at the level of the right dorsolateral prefrontal cortex, a better working memory in RRMS patients was associated with increased FC between right dorsolateral prefrontal cortex and right caudate nucleus and bilateral paracingulate gyrus (106).

Main Findings

- Altered SC and FC of the frontoparietal networks.
- Altered FC of the default mode network.

Long-Term Memory

It represents the ability to learn new information and recall them at a later time (3). Long-term memory is tested by Selective Reminding Test (SRT) (54), California Verbal Learning Test, and Brief Visuospatial Memory Test, Revised (BVMT-R) (4, 54). Impairment in this cognitive domain in MS has a prevalence of 40 to 65% (3).

Structural Connectivity

Hippocampus is the key region of memory in the human brain (107, 108). In CIS and RRMS patients, a decrease in SC, expressed by reduced FA and increased axial diffusivity, along perforant pathways, which connect entorhinal cortex to hippocampus, was found in those patients with memory impairment (109). In another study on RRMS patients assessing tractography-derived hippocampal memory network, worse memory performance was associated with reduction in various SC measures [network efficiency, right hippocampus nodal strength, streamline count, and communicability (i.e., efficiency of the information spread) across network] at the level of the medial temporal lobe, thalamus, insula, and occipital cortex (110).

Functional Connectivity

Altered hippocampal FC is also important for long-term memory deficit in MS. Indeed, RRMS patients with impairment in this cognitive domain showed, compared to healthy controls, decreased FC on the left hemisphere between hippocampus and various cortical regions (superior frontal gyrus, precuneus, posterior cingulate cortex lateral occipital gyrus, angular gyrus) (109) and, compared to memory-preserved MS patients, both increased FC between left hippocampus and right supramarginal gyrus and decreased FC between left hippocampus and right temporo-occipital fusiform/lingual gyrus (109). In another study on RR and SPMS, increased FC in the right posterior hippocampus turned out to be the best correlation of long-term memory impairment (111). Lower dynamic FC of the right hippocampus, in addition to higher static FC of this structure with the rest of the brain, was also able to explain an additional 13% of variance (24% in total) in worse long-term memory in RR and SPMS (112). Following a training with a modified Story Memory Technique in an MS population including different phenotypes (RR, SP, and PP), improvement in long-term memory correlated with increased FC between left hippocampus and cortical regions involved in visual memory and hubs of the default mode network (113). PPMS patients showed increased FC, assessed with seed-based approach, between the cerebellar lobule VIIb and right precentral gyrus, correlating with worse long-term memory measured by BVMT (114). Furthermore, this cerebellar FC reorganization was partially independent from cerebellar atrophy and was probably expression of a maladaptive functional rewiring (114).

SC–FC Coupling

In patients with CIS, stronger structural–functional coupling correlated with worse long-term memory, measured by the SRT-consistent long-term retrieval, suggesting the presence of an exhaustion of functional compensation to structural damage during the early MS stage (70).

Main Findings

- Altered SC and FC in the hippocampus

TABLE 1 | Summary of the main findings from MRI studies in MS patients showing, for each impaired cognitive domain, structural connectivity (SC) damage and functional connectivity (FC) alterations at both global and local levels (when present).

	Main findings in patients with CI (compared with HC and/or patients without CI)	
Impaired cognitive domain	**SC damage**	**FC alterations**
Global cognition	Global ↓ Local efficiency and nodal strength (in 170 RR and 18 SPMS) (61) ↓ Network efficiency (in 133 RRMS) (63)	Global ↓ Mean network degree, global efficiency and hierarchy, ↑ path length, and assortativity (in 45 BMS, 121 RR, and 80 SPMS) (64) Local ↓ Frontoparietal network bilaterally (in 15 RRMS) (65) ↓ Dorsal attention and default mode networks (in 15 RRMS) (66) ↑ FC between thalamic subregions and temporal regions (in 136 RR, 42 SP, and 9 PPMS) (67) ↑ Default mode and frontoparietal networks with the rest of the brain (in 243 RR, 53 SP, and 36 PPMS) (5) ↓ FC: between dorsal attention network and the insula and occipital cortex, between executive control network and the insula and right frontoparietal network, between dorsal attention network and right frontoparietal network (in 13 PPMS) (69)
Attention	Global ↓ Efficiency and clustering coefficient (in 32 RRMS) (72) Local ↓ Integrity of the frontoparietal network bilaterally (in 66 RR and 6 SPMS) (74) ↑ FA along connections from cingulate, frontal and occipital cortices (in 66 RR and 6 SPMS) (74)	Global ↑ Static and dynamic FC (in 25 RRMS) (75) Local ↓ FC in the dorsal attention network and ↑ FC in the ventral attention network during a visual attention task (in 23 RRMS) (76) ↑ FC between frontoparietal network and the rest of the brain (both peripheral and nonhub regions) (in 243 RR, 53 SP, and 36 PPMS) (68)
Information processing speed	Global ↓ Rich-club organization (in 32 RRMS) (78) ↓ Efficiency and clustering coefficient (in 58 RR, 36 SP, and 28 PPMS) (73) ↑ Modularity coefficient (in 19 CIS) (80) Local ↓ Module efficiency within visual network, between visual and deep GM networks and between default mode and frontoparietal networks (in 32 RRMS) (45) ↓ FA-weighted global efficiency of the default mode network, between visual and deep GM networks, and between default mode and frontoparietal networks (in 68 RRMS) (81)	Global ↓ FC at whole-brain level and of the default mode and frontoparietal networks with the rest of the brain (in 83 RR, 31 SP, and 16 PPMS) (82) Local ↓ Effective connectivity from right to left frontoparietal network during a processing speed task (in 16 RR, 3 SP, and 1 PPMS) (83) ↓ FC within default mode network between medial prefrontal and frontal pole regions facilitates performance stability (in 18 RRMS) (84) FC within default mode and salience networks and ↓ FC in the left frontoparietal network (in 40 RR and 25 SPMS) (86) ↑ FC within deep GM and between deep GM and cortex (in late 243 RR and 53 SPMS) (87)
Executive control	Global ↓ Nodal strength within sensorimotor, dorsal attention, left frontoparietal, and default mode networks (in 72 RRMS) (74) Local ↓ Strength in the frontoparietal networks, deep GM and insula (in 33 RRMS) (92) ↓ FA in supratentorial projection and limbic association tracts (in 30 SPMS) (93)	Global ↓ Interplay between dynamic and stationary FC (in 46 RRMS) (94) Local ↓ FC from left posterior parietal to dorsal anterior cingulate (in 18 BMS) (95) ↑ FC from right to left insula (in 33 SPMS) (95) ↓ Effective connectivity from left ventromedial prefrontal cortex to right frontoparietal network, ↑ effective connectivity from left dorsolateral prefrontal cortex to right frontoparietal network (in 14 PPMS) (97)
Working memory	Local ↓ FA along left superior longitudinal fascicle (in 23 RRMS) (102)	Global ↑ Whole-brain functional modularity (in 8 CIS and 8 RRMS) (104) ↑ Flexibility of interhemispheric dynamic FC between temporal regions, anterior cingulate gyrus, and parietal regions (in 25 RRMS) (75) Local ↑ FC between default mode network components (in 5 juvenile MS after cognitive training) (105)

(Continued)

TABLE 1 | Continued

Impaired cognitive domain	Main findings in patients with CI (compared with HC and/or patients without CI)	
	SC damage	**FC alterations**
	↓ Efficiency in the frontoparietal network (in 91 RR and 11 SPMS) (103)	↑ FC between anterior cingulate cortex and right middle frontal gyrus, between anterior cingulate cortex and right inferior parietal lobule (in 17 RRMS after cognitive rehabilitation) (96)
Long-term memory	Local ↓ SC between entorhinal cortex and hippocampus (in 16 CIS and 15 RRMS) (109) ↓ SC measures (efficiency, strength, streamline count, and communicability) in the hippocampal network (in 71 RRMS) (110)	Local ↓ FC between left hippocampus and various cortical regions (superior frontal gyrus, precuneus, posterior cingulate cortex, lateral occipital gyrus, angular gyrus) ↑ FC between left hippocampus and right supramarginal gyrus ↓ FC between left hippocampus and right temporo-occipital fusiform/lingual gyrus (in 15 RRMS) (109) ↑ FC on the right posterior hippocampus (in 53 RR and 11 SPMS) (111) ↑ FC between the cerebellar lobule VIIb and right precentral gyrus (in 29 PPMS) (114) ↓ Dynamic FC of the right hippocampus,and ↑ static FC of the right hippocampus with the rest of the brain (in 30 RR and 8 SPMS) (112)

BMS, benign MS; CI, cognitive impairment; CIS, clinically isolated syndrome; GM, gray matter; HC, healthy control; MS, multiple sclerosis; RR, relapsing–remitting; SP, secondary progressive; PP, primary progressive.

TABLE 2 | Findings of SC damage and FC alterations of the frontoparietal network across cognitive domains in MS.

Cognitive domain	Connectivity type	Connectivity findings
Attention	SC	Decreased nodal strength
	FC	Increased FC
Information processing speed	SC	Decreased communication efficiency between frontoparietal and default mode networks
	FC	Increased FC
Executive control	SC	Decreased nodal strength
	FC	Extra effective connectivity to the right frontoparietal network
Working memory	SC	Decreased global and local efficiency
	FC	—
Long-term memory	SC	—
	FC	—

SC, structural connectivity; FC, functional connectivity.

Table 1 summarizes the SC and FC substrates of the different cognitive domains in MS patients.

Table 2 summarizes the findings of altered SC and FC of frontoparietal network across cognitive domains.

Cognitive Reserve

Cognitive reserve, which reflects the ability to cope with disease-related CI, is thought to explain in MS the incomplete relationship between brain disease and cognitive status (115, 116).

Structural Connectivity

Only recently, SC has been used to investigate cognitive reserve in MS. In a study, a moderate correlation between higher cognitive reserve index and more preserved graph measures of SC (nodal strength, global and local efficiency, cluster coefficient and transitivity) across the brain was observed in RR and SPMS patients with CI but not in those with preserved cognition, a finding that highlights the important protective role of cognitive reserve (117).

Functional Connectivity

A negative relationship between higher cognitive reserve index and lower FC within salience network and occipital regions was observed in RRMS (118). Moreover, RRMS patients with higher premorbid verbal intelligence, a proxy for cognitive reserve, exhibited preserved whole-brain FC despite progressive GM atrophy, stressing the role of preserved FC for a high level of cognitive reserve despite structural damage (119).

Cognitive Phenotypes

The characterization of MS cognitive phenotypes may represent a step toward a better knowledge of the CI pathogenesis and personalized treatment (8). To date, there is no study assessing SC or FC in different MS cognitive phenotypes.

FUTURE DIRECTIONS

New diffusion MRI techniques, such as DKI, DSI, HARDI, and NODDI, should be considered when assessing in MS the relevance toward CI of disconnection in brain regions with crossing fibers (21). Moreover, as MS lesions may affect the tractography-derived reconstruction of WM fibers, they need to be taken better into account. While traditional DTI-based fiber tracking may underestimate the effect of MS

lesions on WM tracts, novel methods such as constrained spherical deconvolution–based fiber tracking (120, 121) and convex optimization modeling for microstructure informed tractography (122) were able to perform a more adequate WM fiber tracts reconstruction in the MS lesional brain (121, 122), thus providing a more reliable assessment of SC (122). Finally, data-driven methods for extracting structural networks, such as ICA and NMF, have rarely been used in the MS field. These methods provide a "soft" parcellation of the brain, where each voxel can contribute to build up multiple structural networks, thus being more sensitive to subtle pathology, whereas for "hard" parcellation, each voxel is uniquely assigned to a single structural network (31).

The field of FC appears fractionated because of the different analysis approaches, and this limits the replication and clinical translation of the various findings (35). In order to improve the clinical impact of FC, it is recommended for subsequent analysis and interpretation following a pipeline of "brain representation," including both a spatial definition of brain units and a summary measure representing their different features (35).

To our knowledge, no study has ever assessed SC and FC in different cognitive MS phenotypes. Future studies in this field would help overcome the heterogeneity of CI in MS and better characterize cognitive groups with impairment in single or multiple domains (54).

Reorganization of both altered SC and FC, whether "compensatory" or "maladaptive," is an important characteristic of MS (38, 123). However, evidence on cognition-related connectivity abnormalities in MS mostly derives from cross-sectional studies, and thus, it is difficult to claim whether such abnormalities may or may not be beneficial for cognitive performance of MS patients (123). Large prospective longitudinal studies of multimodal MRI are needed in MS in order to reveal relationships between worsening CI and changes over time in specific brain structures and functions (54).

"Fusion" methods (124), by considering the brain as a unified system, are able to simultaneously map alterations across different MRI modalities and include unsupervised multivariate methods such as independent component analysis (124), canonical correlation analysis, partial least-squares regression (125), and multilayer brain networks (126, 127). Such methods may be useful in shedding light on the joint mechanisms of altered SC and FC reorganization underlying CI in MS.

CONCLUSIONS

In recent years, studies on SC and FC contributed to the understanding of MS-related CI. However, further studies are needed to make these abnormalities more easily interpretable in the research setting and above all useful in clinical practice, by taking into account the use of standardized pipelines and the possible bias introduced by MS lesions. Finally, longitudinal multimodal MRI studies may shed light on the changing associations between concurrent pathogenic mechanisms and MS-related CI.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Cognitive Decline in Multiple Sclerosis Is Related to the Progression of Retinal Atrophy and Presence of Oligoclonal Bands: A 5-Year Follow-Up Study

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Background: Brain atrophy, which is associated with cognitive impairment and retinal nerve fiber layer (RNFL) atrophy, is the main biomarker of neurodegeneration in multiple sclerosis (MS). However, data on the relationship between inflammatory markers, such as oligoclonal bands (OCBs) in the cerebrospinal fluid (CSF), and cognition, RNFL atrophy, and brain atrophy are scarce. The aim of this study was to assess the influence of RNFL thickness, brain atrophy markers, intrathecal OCBs, and the immunoglobulin G (IgG) index on cognitive decline over a 5-year period in patients with MS.

Methods: This prospective, single-center, observational cohort study included 49 patients with relapsing MS followed up over 5 years. At baseline, the patients underwent brain magnetic resonance imaging (MRI). Cognitive evaluation was performed using the Brief International Cognitive Assessment for MS (BICAMS), and RNFL thickness was assessed using optical coherence tomography (OCT). OCBs and IgG levels in the CSF were evaluated at baseline. The BICAMS, OCT, and MRI findings were re-evaluated after 5 years.

Results: A significant reduction in information processing speed, visual learning, temporal RNFL thickness, the Huckman index, and third ventricle mean diameter was found in all 49 patients with relapsing MS over the observation period ($p < 0.05$). Of the patients, 63.3% had positive OCBs and 59.2% had elevated IgG indices. The atrophy of the temporal segment and papillomacular bundle and the presence of OCBs were significantly related to a decline in information processing speed in these patients ($p < 0.05$). However, brain atrophy markers were not found to be significant on the general linear models.

Conclusions: RNFL atrophy and the presence of OCBs were related to cognitive decline in patients with MS over a 5-year follow-up period, thereby suggesting their utility as potential biomarkers of cognitive decline in MS.

Keywords: multiple sclerosis, cognition, BICAMS, OCT, oligoclonal bands, brain atrophy

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease of the central nervous system (CNS) (1) that leads to demyelination and diffuse neurodegeneration in both the brain and spinal cord gray matter and white matter (1, 2). The course of the disease is usually relapsing-remitting from onset (1, 3). Studies have also shown the involvement of both inflammatory and neurodegenerative processes from the early stages of the disease (2, 4, 5). However, it remains unknown whether early degeneration is an independent process in MS or whether it is secondary to inflammation (2, 5). Inflammation in MS is more obvious and can be easily assessed, documented, and monitored in patients. In contrast, neurodegeneration is more difficult to assess and monitor, especially in the early stages of the disease (5).

Understanding the mechanism and causes of neurodegeneration in MS may be fundamental to developing therapies that can help halt this process and presumably prevent the progression of disability (2, 3). Brain atrophy assessed using magnetic resonance imaging (MRI) may be a biomarker for early neurodegeneration and may help predict the prognosis and disease course. Nevertheless, the measurement of atrophy on MRI in routine clinical practice remains a hurdle (6, 7). The identification of sensitive and accessible markers of and diagnostic tools for neurodegeneration may help us understand the relationship between these markers and may facilitate the development of easy-to-use and low-cost tools for exploring the pathophysiology of neurodegeneration in MS (2–4).

Cognitive impairment in MS reflects the underlying inflammatory and neurodegenerative pathological features of the disease (8). It is present in up to 50–70% of patients with MS and significantly lowers their quality of life (8, 9). The most frequently observed cognitive problems include deficits in information processing speed, episodic memory, complex attention, and executive function (8, 10, 11). The severity of cognitive impairment varies considerably among individuals and can be observed even in the early stages of the disease (12, 13). Brain imaging studies have demonstrated that cognitive impairment in MS is related to the loss of brain volume or brain atrophy, which is an important sign of neurodegeneration (8, 10). Cognitive impairment and brain atrophy have been classically considered as features that present in the advanced stages of the disease (14). However, numerous studies have demonstrated that both cognitive impairment and brain atrophy may occur in the early stages of the disease and even in clinically and radiologically isolated syndromes (15, 16).

Optical coherence tomography (OCT) measurements of the macular ganglion cell layer and retinal nerve fiber layer (RNFL) have been proposed as biomarkers of axonal damage in MS (17). Recently, retinal OCT has been used as a sensitive and practical alternative to MRI for the evaluation of neurodegeneration in MS (17, 18). However, studies have demonstrated a strong relationship between cognitive impairment across various cognitive domains and RNFL atrophy even in patients without MS-related optic neuritis (19–21). Some studies also indicate that RNFL thickness and cognition could be sensitive biomarkers that can be used for discriminating relapsing and progressive forms of the disease (21, 22). RNFL thickness may be associated with brain atrophy and cognitive impairment; therefore, OCT may be useful in assessing CNS neurodegeneration in MS (23, 24).

The presence of oligoclonal bands (OCBs) in the cerebrospinal fluid (CSF) or an elevated immunoglobulin G (IgG) index in patients with MS supports the diagnosis. Persistent intrathecal inflammation, demonstrated by the presence of OCBs in the CSF (25), is one of the hallmarks of MS in up to 95% of patients (25, 26). Previous research demonstrated that the presence of CSF-OCBs in patients with MS tends to be related to widespread cognitive changes, especially worse visual memory (27) and larger periventricular lesion area on MRI (28). However, data on the relationship between inflammatory markers, such as CSF-OCBs or elevated IgG indices, and neurodegenerative markers, such as brain atrophy markers or RNFL thickness, in MS are limited (27).

The aim of this study was to assess the impact of neurodegenerative markers, such as RNFL thickness and brain atrophy, as well as inflammatory markers, such as intrathecal OCBs and the IgG index, on the cognitive decline in patients with MS over a 5-year follow-up period.

MATERIALS AND METHODS

This prospective, single-center, observational cohort study was conducted at Vilnius University Hospital Santaros Klinikos, Lithuania. Patients were enrolled and assessed between 2012 and 2019. All patients signed an informed consent form, and the study was approved by the appropriate institutional review board. The inclusion criteria were as follows: age between 18 and 60 years, presence of relapsing-remitting MS, and absence of relapse and/or steroid treatment at least 30 days before the enrollment assessment and during the follow-up assessment. All patients were on stable-disease-modifying therapy at least 3 months before the assessment, and none had a history of MS-associated optic neuritis. The exclusion criteria were the presence of primary or secondary progressive MS, neurological disorders other than MS, any vision or hearing problems that could influence performance on the tests, and optic neuritis during the observation period.

After providing signed written informed consent, all the patients underwent physical and neurological examinations, neuropsychological assessment using the Brief International Cognitive Assessment for MS (BICAMS), ophthalmological examination using OCT, and brain MRI. The same evaluations were repeated 5 years (±14 days) later. The changes from baseline to the follow-up visit were calculated for all assessments.

All patients with MS were diagnosed according to the McDonald criteria by a neurologist at the Vilnius Multiple Sclerosis Center (29, 30). Neurological disability was assessed

Abbreviations: OCB, oligoclonal band; BICAMS, Brief International Cognitive Assessment for Multiple Sclerosis; EDSS, Expanded Disability Status Scale; SDMT, Symbol Digit Modalities Test; BVMT-R, Brief Visuospatial Memory Test Revised; CVLT-II, California Verbal Learning Test, Second Edition; PMB, Papillomacular Bundle; BCR, Bicaudate Ratio.

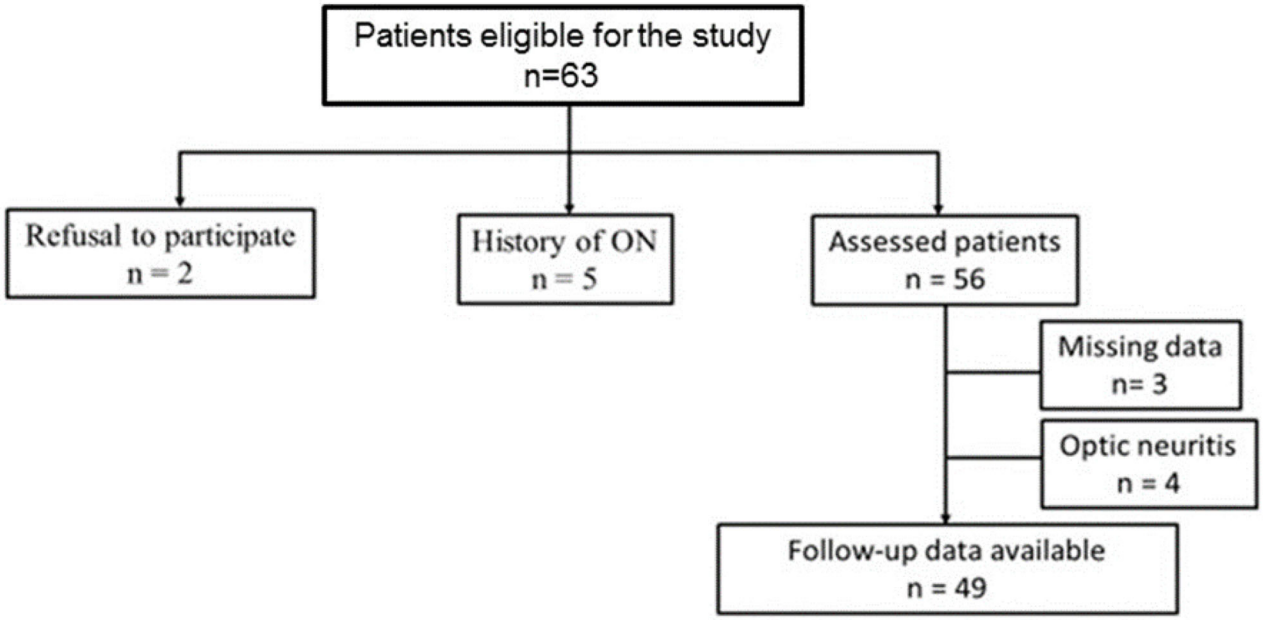

FIGURE 1 | Flowchart illustrating patient selection. ON, optic neuritis.

using the Expanded Disability Status Scale (EDSS) (31). The patients also underwent a lumbar puncture for evaluating the CSF-OCBs and IgG index at baseline.

BICAMS

All the patients were examined by the same neurologist, and the tests were administered in the same sequence: the Symbol Digit Modalities Test (SDMT) to evaluate the information processing speed; the Brief Visuospatial Memory Test Revised (BVMT-R), i.e., the first three recall trials to evaluate visual learning and memory; and the California Verbal Learning Test, Second Edition (CVLT-II), i.e., the first five trials to evaluate verbal learning and memory (32–35). The baseline and follow-up assessments were performed by the same neurologist. Different versions of the BICAMS test were used during the baseline and follow-up assessments.

OCT

OCT was performed on both eyes of each patient by using a spectral-domain OCT device (Spectralis, Heidelberg Engineering, Heidelberg, Germany), and the images were evaluated by the same ophthalmologist. RNFL thickness was measured using the RNFL-N axonal protocol with three 3.4-mm-diameter circular scans. The RNFL Spectralis protocol generates maps with four quadrants (superior, inferior, nasal, and temporal) and six sector thicknesses (superonasal, nasal, inferonasal, inferotemporal, temporal, and superotemporal); it also measures the thickness of the papillomacular bundle (PMB), the nasal-to-temporal ratio, and the average thickness.

MRI

Brain MRI with gadolinium enhancement was performed in all patients by using a Siemens Aera 1.5 T MRI scanner (Siemens, Munich, Germany). MRI assessment included the following sequences: T1 (repetition time, 526 ms; echo time, 14 ms), T2 (repetition time, 4,110 ms; echo time, 105 ms), and fluid-attenuated inversion recovery (FLAIR) T2 (repetition time, 9,000 ms; echo time, 122 ms). A radiologist who was blinded to the patient's clinical data calculated the linear measures of brain atrophy. To evaluate brain atrophy, the Huckman index (sum of the greatest and smallest distances between the frontal horns), third ventricle width, and bicaudate ratio (BCR) were measured. The BCR was measured on a FLAIR axial image, where the heads of the caudate nuclei were best visible and closest to each other. The BCR was determined as the minimum intercaudate distance divided by the distance between the outermost parts of both the hemispheres measured along the same line.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 23.1 (IBM Corp., Armonk, NY, USA). Continuous variables were reported as medians and ranges or means and standard deviations, while categorical variables were reported as absolute numbers and percentages of total patients. The normal distribution of the data was verified using the Shapiro–Wilk test. Student's *t*-test was used to compare the means between the two groups (baseline and follow-up assessments). The chi-square test was used for categorical variables. General linear regression was used to assess the relationship between the change in cognitive functions over 5 years (dependent variable) and the following clinical and demographic factors as explanatory variables: the

TABLE 1 | Clinical and demographic characteristics of the patients.

Demographic and clinical variables	*N*	%
Sex		
Female	37	75.5
Age (years)	47.3 ± 11.1	–
Disease duration (years)	11.4 ± 4.6	–
Education (years)	13.7 ± 3.2	–
EDSS		
Baseline assessment	2.8 ± 1.1	–
Follow-up assessment*	4.0 ± 1.4	–
Nonocular relapses**	2.2 ± 2.1	–
OCBs		
Positive	31	63.3
IgG index		
Elevated***	29	59.2

EDSS, expanded disability status scale; OCBs, oligoclonal bands; IgG index, immunoglobulin G index.
**Follow-up was performed 5 years later.*
***Relapses were assessed over 5 years (from baseline assessment up to the follow-up assessment).*
****The IgG index was considered elevated when it was more than 0.77.*

TABLE 2 | Cognitive scores at baseline and follow-up in patients with MS.

Test	Baseline assessment	Follow-up assessment*	*p***
SDMT	44.5 ± 12.6	40.3 ± 12.6	**<0.001**
BVMT-R	24.7 ± 6.1	23.1 ± 7.2	**<0.05**
CVLT-II	59.5 ± 9.2	57.4 ± 11.5	>0.05

SDMT, symbol digit modalities test; BVMT-R, brief visuospatial memory test revised; CVLT-II, california verbal learning test, Second Edition.
**Follow-up was performed 5 years after the baseline assessment.*
***Student's t-test for paired samples. Bold values indicate significant differences or indicators.*

change in RNFL thickness over 5 years, the change in brain atrophy markers over 5 years, the presence of OCBs, the IgG index, disease duration, age, and sex. The dependent variables in the models were the changes in SDMT, BVMT-R, and CVLT-II over 5 years. The independent variables (regressors) were the changes in different segments of the RNFL over 5 years in both eyes; the changes in brain atrophy markers on brain MRI (third ventricle width, Huckman index, or bicaudal score) over 5 years; the difference in the EDSS score between the baseline and follow-up assessments; and the IgG index, the presence of OCBs, age, sex, and disease duration at baseline. A value of $p < 0.05$ was considered significant.

RESULTS

Patients

Sixty-three patients were enrolled in this study. The 5-year follow-up data were available for 49 patients (77.8%) (**Figure 1**).

All patients had relapsing-remitting MS. The demographic and clinical characteristics of the patients are listed in **Table 1**.

CSF Assessment

Of 49 patients, 63.3% had positive OCBs and 59.2% had elevated IgG indices in the CSF. Positive OCBs and elevated IgG indices did not differ according to sex ($\chi^2 = 0.079$, $p > 0.05$ and $\chi^2 = 0.843$, $p > 0.05$, respectively), age ($p > 0.05$), and disease duration ($p > 0.05$). The severity of disability was assessed using the EDSS at baseline, and the changes in EDSS scores between the baseline and follow-up assessments did not differ between patients with positive and negative OCBs ($p > 0.05$), as well as between patients with elevated and lower than normal IgG indices ($p > 0.05$). The incidence of positive OCBs and elevated IgG indices was similar in patients (63.3 and 59.2%, respectively); however, no relationship was found between positive OCBs and elevated IgG indices ($\chi^2 = 0.993$, $p > 0.05$).

Cognitive Dynamics in Patients With MS

The scores of the SDMT and BVMT-R were significantly lower during the follow-up assessment than at the baseline assessment, while the CVLT-II scores did not differ between the baseline and follow-up assessments (**Table 2**).

RNFL Thickness Determined Using OCT

The average RNFL thickness in the temporal, nasal, inferotemporal, and inferonasal segments and the overall global average thickness were significantly lower in both eyes at the follow-up assessment ($p < 0.05$), while the average thickness of the PMB was lower in the right eye and the thickness of the superotemporal segment was lower in the left eye. The OCT results are presented in **Table 3**.

Linear Measures of Brain Atrophy

The Huckman index and third ventricle width were significantly lower during the follow-up assessment than at the baseline assessment. However, the BCR did not differ between the baseline and follow-up assessments (**Table 4**).

Relationship of Disease Characteristics and Biomarkers of Neurodegeneration and Inflammation to Cognitive Decline

A general linear model was used to assess the relationship of the changes in RNFL thickness, brain atrophy markers, EDSS scores, OCBs, IgG index, and disease characteristics (age, sex, and disease duration) to the changes in cognitive domains over 5 years. The dependent variables in the models were the changes in SDMT, BVMT-R, and CVLT-II scores over 5 years. The independent variables (regressors) were the changes in different segments of the RNFL over 5 years, which were assessed as the changes in the mean values for both eyes; the changes in brain atrophy markers on brain MRI (third ventricle width, Huckman index, or bicaudal score) over 5 years; the differences in the EDSS scores between the baseline and follow-up assessments; and the IgG index, presence of OCBs, age, sex, and disease duration at baseline (**Table 5**). The decline in information processing speed over 5 years in patients with relapsing MS was explained by the RNFL thickness in the temporal segment or PMB in both eyes as well as the CSF-OCBs.

TABLE 3 | Changes in RNFL thicknesses in patients with MS at the baseline and follow-up assessments.

Segment	Right eye, $\Delta RNFL_{B-5}$* ± SD**	p***	Left eye, $\Delta RNFL_{B-5}$* ± SD**	p***	Both eyes, $\Delta RNFL_{B-5}$* ± SD**	p***
T	2.2 ± 4.1	**<0.001**	1.6 ± 4.9	**<0.05**	1.9 ± 3.7	**<0.001**
N	4.2 ± 4.9	**<0.001**	4.4 ± 5.5	**<0.001**	4.3 ± 4.3	**<0.001**
TS	1.1 ± 5.1	>0.05	1.8 ± 5.9	**<0.05**	1.4 ± 4.7	**<0.05**
TI	4.5 ± 6.5	**<0.001**	3.9 ± 7.4	**<0.001**	4.2 ± 5.4	**<0.001**
NS	−0.2 ± 5.0	>0.05	0.9 ± 6.0	>0.05	0.3 ± 4.2	>0.05
NI	4.3 ± 6.8	**<0.001**	4.0 ± 7.7	**<0.001**	4.2 ± 5.6	**<0.001**
PMB	1.9 ± 3.6	**<0.001**	0.7 ± 4.9	>0.05	1.3 ± 3.3	**<0.05**
G	2.8 ± 3.1	**<0.001**	2.7 ± 4.4	**<0.001**	2.8 ± 3.3	**<0.001**

RNFL, retinal nerve fiber layer; SD, standard deviation; T, temporal; N, nasal; TS, superotemporal; TI, inferotemporal; NS, superonasal; NI, inferonasal; PMB, papillomacular bundle; G, global.

**Change from the baseline to follow-up assessments: the mean of delta.*

***Standard deviation of delta.*

****Student's t-test for paired samples. Bold values indicate significant differences or indicators.*

DISCUSSION

Cognitive impairment, RNFL thickness, and brain atrophy are markers of neurodegeneration in MS (21, 29, 36, 37), whereas positive OCBs and elevated IgG indices in the CSF are markers of inflammation (25, 26). MRI was long considered the gold standard for monitoring the degenerative component of MS (6, 7). Thereafter, RNFL thickness and cognition were recognized as biomarkers of neurodegeneration (14, 17, 18). The presence of CSF-OCBs in patients with MS is supportive of the diagnosis (29, 30), even though the relationship between the patient's clinical and cognitive features has not been thoroughly examined. In our study, positive OCBs were detected in 63.3% of patients and elevated IgG indices were detected in 59.2%. CSF biomarkers such as OCBs and elevated IgG indices were not correlated with each other. Nevertheless, both are markers of inflammation and both are supportive of a diagnosis of MS (30). Previously published data regarding the correlation between the presence of OCBs and elevated IgG levels differ among studies; while some studies have reported positive correlations (38), others have not found any relationship (39–41). Moreover, in most patients with MS, when the number of OCBs is >2, no linear association is observed between CSF IgG levels and the number of OCBs (39, 40). The absence of such a correlation is possible because OCBs reflect the production of several monoclones, while the IgG index is a general indicator of enhanced autoimmune response. In our study, we did not find a correlation between the presence of OCBs and the IgG index.

We investigated whether cognitive decline over 5 years in patients with relapsing MS can be explained using neurodegenerative and inflammatory markers such as OCBs and IgG indices in the CSF. In the recently published revision of the McDonald diagnostic criteria, the detection of oligoclonal IgG bands in the CSF has regained importance (30). Therefore, we decided to assess the impact of inflammatory markers on cognitive decline. We found that among the biomarkers of neurodegeneration and neuroinflammation, RNFL thickness in the temporal segment, PMB thickness in both eyes, and the presence of OCBs were explanatory variables indicating a decline in information processing speed in patients with MS.

TABLE 4 | Brain atrophy markers at the baseline and follow-up assessments in patients with MS.

Brain atrophy marker	Baseline assessment	Follow-up assessment*	p**
HI	49.3 ± 7.3	52.0 ± 8.3	**<0.001**
TVW	4.7 ± 1.9	6.3 ± 2.1	**<0.001**
BCR	0.1 ± 0.03	0.1 ± 0.04	>0.05

HI, huckman index; TVW, third ventricle width; BCR, bicaudate ratio.

**Follow-up was performed 5 years after the baseline assessment.*

***Student's t-test for paired samples. Bold values indicate significant differences or indicators.*

Many studies have provided data on one particular cognitive measure, i.e., the SDMT, which is considered particularly sensitive to the decrease in information processing speed that is commonly seen in MS (42, 43). Owing to its high reliability, validity, sensitivity, and specificity, the SDMT has demonstrated superiority over other cognitive tests for MS in recent years (43). Our findings are consistent with previously published data (42–44), and the SDMT was the only cognitive test in which the results were related to other markers of neurodegeneration and inflammation in our cohort. Our data also confirmed the association between cognitive function and RNFL thickness. In particular, we found that the average thickness in the temporal segment and PMB in both eyes was the most important OCT measure related to cognitive decline in our patients. During the past decade, OCT has developed into a sensitive method for imaging neurodegeneration in MS (17, 45). Studies have demonstrated that lower average

TABLE 5 | Regression models that explain the cognitive decline over 5 years in patients with MS.

Dependent variable	Regression model	*R2*	*p* (R^2; coefficients)
$\Delta SDMT_{B-5}$	$-3.1 - 1.0 \times (\Delta RNFL\_T_{B-5}) + 3.3 \times CSF\_OCBs$	0.599	<0.01
$\Delta SDMT_{B-5}$	$-8.8 - 1.1 \times (\Delta RNFL\_PMB_{B-5}) + 4.4 \times CSF\_OCBs$	0.480	<0.01

^{R}2, coefficient of determination; $\Delta SDMT_{B-5}$, difference in SDMT results between the baseline and follow-up assessments; SDMT, symbol digit modalities test; $\Delta RNFL\_T_{B-5}$, the difference in temporal segment thickness assessed as the change in the mean of the value for both eyes between the baseline and follow-up assessments; $\Delta RNFL\_PMB_{B-5}$, the difference in papillomacular bundle thickness assessed as the change in the mean of the value for both eyes between the baseline and follow-up assessments; CSF_OCBs, oligoclonal bands in the cerebrospinal fluid.

temporal RFNL thickness correlates with a more active disease course, higher EDSS at the time of assessment, and greater EDSS score increase over time (37, 46). Correlations were also found between RNFL thickness and performance on some tests of cognitive function in patients with MS, particularly the SDMT (46, 47). Our results are in line with these previously published data showing that the SDMT score and RNFL thickness in the temporal segment are significant cognitive and ophthalmological indicators of neurodegeneration in MS (46, 47).

The limitations of our study were the relatively small sample size and the lack of a control group. However, we did not identify any controlled study in which a comparison group was used to assess the presence of OCBs and IgG indices in the CSF or in which the patients were followed up for a long duration of 5 years.

Another innovative aspect of our work was the combined analysis of inflammatory OCBs, neurodegeneration-related RNFL thickness, and cognition. The dependence of cognition on the presence of OCBs and RNFL thickness has not been previously investigated. We found a relationship between both neurodegenerative and inflammatory markers and information processing speed. RNFL thickness in the temporal segment, PMB thickness, and the presence of OCBs could be considered biomarkers in the diagnostic workup for MS. We did not detect a significant influence of any other RNFL segment thickness or brain linear measurement on cognition in our cohort of patients with MS. Our results confirm that the BICAMS and OCT measure different aspects of neurodegeneration and that the thinning of the RNFL is a potential biomarker for cognitive disability in MS (23, 47), because we found that cognitive decline may be predicted not only by markers of degeneration but also by markers of intrathecal inflammation. These results imply that both the thinning of the RNFL and the presence of CSF-OCBs are feasible biomarkers for cognitive decline in MS.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Lithuanian Bioethics Committee approved the study in 2011 (2011-01-27 No.: L-12-01/2), the permission to continue the study was granted by the Lithuanian Bioethics Committee in 2018 (2018-02-22 No.: 6B-18-41). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NG contributed to the conception and design of the study, acquisition of the data, analysis and interpretation of the data, and drafting of the manuscript. ED contributed to the analysis and interpretation of the data and to the conception and design of the study. RK contributed to the conception and design of the study and the revision of the manuscript. AC contributed to the analysis and interpretation of the data and drafting of the manuscript. RA contributed to the analysis and interpretation of the data and drafting of the manuscript. GK contributed to the conception and design of the study, analysis and interpretation of the data, and drafting of the manuscript. All authors discussed the results and contributed to and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.678735/full#supplementary-material

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