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TESI DI LAUREA:

"Cerebellar Contribution to Cognitive Impairment in early stages of Relapsing-Remitting Multiple Sclerosis: a conventional and rs-fMRI study"

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Abbreviations

BOLD	Blood Oxygenation Level Dependent
CCAS	Cerebellar Cognitive Affective Syndrome
CI	Cognitive Impairment
CIS	Clinically Isolated Syndrome
CNS	Central Nervous System
DIR	Double Inversion Recovery
DTI	Diffusion Tensor Imaging
EDSS	Expanded Disability Status Scale
FC	Functional Connectivity
FLAIR	Fluid-Attenuated Inversion Recovery
GML	Gray Matter Lesion
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
NAGM	Normal Appearing Gray Matter
NAWM	Normal Appearing White Matter
PPMS	Primary-Progressive MS
ROI	Region Of Interest
RRMS	Relapsing-Remitting MS
rs-fMRI	Resting-state functional MRI
SDMT	Symbol Digit Modalities Test
SPMS	Secondary Progressive MS
WML	White Matter Lesion

Abstract

Background. The cerebellum is a primary site of Multiple Sclerosis (MS) pathology. Structural and functional MRI studies have demonstrated the role of the posterior cerebellum in cognitive functions. To date, the "Cerebellar Cognitive Affective Syndrome" (CCAS) scale has never been used to test MS-related Cognitive Impairment (CI) and its association with cerebellar involvement.

Objectives. We investigated the association of MRI structural and functional abnormalities of the cognitive cerebellum with CI and tested the role of the CCAS scale in detecting CI in a cohort of very early RRMS patients.

Methods. 37 patients with early RRMS and 4 age- and sex-matched healthy controls (HC) were enrolled in this cross-sectional, exploratory study. Cognitive performances were assessed through BICAMS, D-KEFS ST, and CCAS scale. Using a CCAS scale score cut-off (based on a 50 HC sample), 26/37 (70%) patients were classified as "Normal-CCAS" and 11/37 (30%) as "Impaired-CCAS". All subjects underwent a conventional and resting-state functional MRI (rs-fMRI) protocol. Comparisons between groups were assessed for structural and functional MRI parameters. Moreover, correlations between cognitive test scores and structural-functional MRI parameters were evaluated.

Results. Patients with pathological score on CCAS also showed CVLT-II and D-KEFS ST low scores. A significant reduction in cerebellar volumetric parameters was found in the CCAS-impaired MS group compared to the normal one, albeit whole brain WM and thalamic volumes were also significantly reduced. The rs-fMRI analysis revealed higher functional connectivity (FC) between the cognitive cerebellum and most of the functional brain cortical networks in the CCAS-impaired group compared to the normal one.

Conclusions. Our findings suggest that CI in early RRMS is associated with pathological alterations in both structural and functional MRI parameters. Higher FC between cerebellar-brain networks in CCAS-impaired patients might be the expression of a compensatory hyperactivation of altered cognitive cerebellar connections. Finally, although the CCAS scale has proven able to detect CI in MS patients, its specificity for cerebellar pathology needs to be further investigated.

1. Introduction

1.1. Multiple Sclerosis

Multiple Sclerosis (MS) is the most common chronic inflammatory, demyelinating, and neurodegenerative disease of the Central Nervous System (CNS)[1]. It represents the leading cause of non-traumatic neurological disability in young adults[2].

In 2013 Lublin et al.[3] divided the clinical course of MS into two core phenotypes: relapsing or progressive. Most commonly, the onset is a relapsing form of MS (termed Relapsing-Remitting MS [RRMS]), characterized by transient episodes of neurological dysfunction, followed by periods of partial or complete remission with clinical stability. Over time, relapses usually decrease in frequency, but a gradual worsening supervenes, resulting in uninterrupted and irreversible progression (termed Secondary Progressive MS [SPMS]). Less than 10% experience progression from the onset (termed Primary Progressive MS [PPMS])[3].

Despite these distinctions, all clinical forms of MS appear to reflect the same underlying pathological processes. Although inflammation is typically associated with relapses and neurodegeneration with progression, it is now recognized that both aspects are present in essentially all patients across the entire disease continuum[4].

1.1.1. Relapsing-Remitting Multiple Sclerosis (RRMS)

1.1.1.1. Epidemiology

MS is a significant global health issue, with more than 2.8 million people affected worldwide[2]. Conclusive contributions of the geographical distribution of MS have been published by Kurtzke[5], who divided prevalence into what (on a global scale) would be regarded as high (>30 per 100 000), as found in northern parts of Europe and North America; medium (5–30 per 100 000), as found in southern Europe and southern USA; and low (<5 per 100 000), as found in Asia and South America.

The prevalence of MS has increased since the 1950s, primarily due to longer life expectancy in the affected population[6]. However, this reasoning cannot account for the female preponderance seen in RRMS nor the increase in the female-to-male ratio, which has risen from an estimated 1.4 in 1955 to 2.3 in 2000[7] and now exceeds 3.0 in some populations[8]. This rapid shift suggests environmental factors are involved, potentially in combination with gene-environment interactions. It also implies that a significant portion of MS cases may be preventable by targeting factors that have changed in the modern female lifestyle, such as occupation, cigarette smoking, obesity, birth control, and delayed childbirth[6,8].

RRMS typically manifests between 20 and 40 years of age, but it can occur at any point in life: up to 10% of patients are diagnosed during their childhood or adolescence[9] while others receive the diagnosis in late adulthood. The age of onset appears to influence the sex distribution of RRMS patients (female-to-male ratio at onset: <10 years 1.4, 18-49 years 3.1, 50-59 years 2.3)[10,11].

The higher incidence of RRMS in females might be attributed to sex-based differences in immune function driven by hormonal factors which predispose women to stronger autoimmune responses[12]. In addition, men may have a lower risk of developing RRMS due to the potential neuroprotective effects of testosterone[13]. Conversely, men are more susceptible to developing progressive forms of MS and exhibit a more pronounced neurodegenerative component and disability progression[14].

1.1.1.2. Etiology

MS is a complex, multifactorial disease with an immune-mediated nature [1]. Its precise cause is not yet fully understood. However, it is believed to arise in genetically susceptible individuals, where stochastic events and environmental factors influence the disease penetrance[15].

Although MS is not an inherited disease, genetics plays an important role in its etiology, as evident from the clustering of MS cases within families. People with an affected first-degree relative have a 2-4% risk of developing MS, compared to the general population risk of 0.1%. Additionally, there is a concordance rate of approximately 30% in monozygotic twins[16].

Thanks to genome-wide association studies (GWAS), based on samples assembled from thousands of patients and matched controls, over 200 gene variants associated with MS have been identified. Among these variants, the human leukocyte antigen DRB1*1501 haplotype is the most significant (OR=3). Other genetic loci outside of the HLA region have been implicated in MS. These extra-HLA loci often involve genes related to immune pathways, including receptors for interleukin-2 (IL-2) and interleukin-7 (IL-7), interferons (IFNs), and tumor necrosis factor receptors (TNFRs). These findings provide further support for the understanding that MS is primarily an immune-mediated disease [17,18].

While genetic susceptibility explains the clustering of MS within families, it alone cannot account for the geographical distribution of the disease or the changes in MS risk associated with migration. These observations suggest the involvement of significant environmental factors. Among these, vitamin D status, obesity in early life, infection with the Epstein-Barr virus (EBV), and cigarette smoking represent the most consistent environmental predictors of MS risk[19,20].

Infectious agents, such as the Epstein-Barr virus (EBV), have been proposed as potential triggers for the onset of MS. EBV, in particular, has shown the most consistent association with MS, with nearly 100% of MS patients testing positive for EBV antibodies[21]. Understanding the exact role of EBV in the development of MS remains challenging. One proposed mechanism is that the chronic presentation of viral antigens may lead to an autoimmune response through molecular mimicry, where the immune system mistakenly targets self-antigens that resemble viral components [21].

A recent publication by Bjornevik et al.[22] tested the hypothesis of a causal relationship between MS and EBV infection. The study involved a large cohort of over 10 million young adult soldiers in the US army, 955 of whom were diagnosed with MS during their service period. The risk of developing MS was increased 32-fold following EBV infection, and not from other viruses with similar transmission, such as CMV. They observed an increase in serum levels of neurofilament light chain, a biomarker of neuroaxonal degeneration, only after EBV seroconversion, which provides additional support for EBV as the leading risk factor for MS.

Several studies have indicated a potential association between low vitamin D levels and increased risk of developing MS, as well as increased disease activity[23]. This suggests that maintaining normal vitamin D levels throughout the course of the disease may have a protective effect, largely due to the beneficial immuno-modulatory effects of vitamin D. However, there is still a notable lack of conclusive randomized clinical trials that have definitively tested the role of vitamin D supplementation in MS patients [24].

1.1.1.3. Pathology

MS was first identified as a novel disease about 150 years ago by Professor Jean-Martin Charcot. In 1868, during his lectures, he named it *Sclérose en Plaque*. Indeed, the pathological hallmark of MS is the formation of plaques in the CNS, which represent focal areas of demyelination, inflammation, and glial reaction[25].

The heterogeneity of the lesions observed in MS patients further complicates the understanding of the pathogenesis of the disease. Although the anatomical location of White Matter Lesions (WMLs) is associated with specific clinical manifestations of MS, the total volume of these lesions is only moderately correlated with the overall clinical disability and cognitive impairment observed in MS patients. This suggests that other pathophysiological mechanisms come into play, such as the occurrence of Gray Matter Lesions (GMLs) and damage to both Normal-Appearing White Matter (NAWM) and Gray Matter (NAGM)[26,27].

WMLs and GMLs in MS display distinct immunological characteristics. While WMLs exhibit significant immune cell infiltration and can be classified according to their immunological activity, GMLs typically show only minimal infiltration of immune cells [28,29].

WMLs can vary in their location, size, and shape among MS patients, thus several classifications have been proposed[30]. For example, Bö and Trapp[31,32] have divided WMLs into *active*, *chronically active*, and *inactive* lesions. *Active* lesions are hypercellular lesions characterized by relative axonal preservation and massive infiltration of lymphocytes, MHC-II+ cells, and myelin-laden macrophages distributed uniformly throughout the lesion. *Chronically active* lesions also show relative axonal preservation, but myelin-laden macrophages only accumulate at the

edges of the lesion. *Inactive* lesions, in contrast, are hypocellular lesions characterized by substantial axonal and oligodendrocyte loss, astrogliosis, and a lesser infiltration of macrophages, microglia, and lymphocytes.

Lucchinetti and colleagues[33] proposed another categorization of active WMLs based on their pathological profiles. In this regard, four distinct patterns of demyelination have been described:

i) *Type I* lesions are characterized by active demyelination associated with T cell infiltration, activated microglia, and macrophages-dominated inflammation with related products (e.g., tumor necrosis factor α), in the absence of immunoglobulins (Ig) and complement deposition;

ii) *Type II* lesions (the most frequent pattern) are similar to type I lesions but additionally show the deposition of Ig (with pronounced reactivity to myelin degradation products) and complement;

iii) *Type III* lesions also contain an inflammatory infiltrate similar to type I lesions but Ig and complement deposition is absent. In contrast to type I and II lesions, demyelination is not centered around veins and venules; instead, a rim of myelin is preserved around inflamed vessels within the plaque. The lesion borders are ill-defined, showing diffuse spread into apparently normal periplaque WM. Oligodendrocytes apoptosis with a preferential loss of myelin-associated glycoprotein (MAG) is observed;

iv) *Type IV* lesions are extremely rare and associated with non-apoptotic death of oligodendrocytes in a small rim of periplaque WM, creating a sharply demarcated plaque of demyelination with radial expansion.

Whether these different patterns represent different subtypes of WMLs or different stages within the formation of WMLs is still a matter of debate.

Evidence from MRI and pathological studies indicates that the earliest stages of WM demyelination are heterogeneous and evolve over months[33]. This suggests that the initial events leading to demyelination may vary among individuals and possibly within the same individual over time. However, as the lesions progress and become more established, there seems to be a convergence toward a dominant immune-effector mechanism within each person[34].

What determines the long-term fate of a lesion – whether inflammation resolves or "smolders" or whether remyelination occurs – is not well understood[35]. Remyelination refers to the process of restoring myelin around demyelinated axons, and it can result in the formation of *shadow plaques* characterized by partially remyelinated areas within the lesion. The extent of this process is highly variable among patients and depends on many factors, including patients' age, disease duration, lesion location, presence of oligodendrocyte progenitor cells, and axonal integrity. Substantial remyelination is frequently observed in the early stages of RRMS and younger individuals, while it is less common or even absent in progressive forms [36–38].

In addition to focal lesions, NAWM often exhibits signs of diffuse inflammation and neuroaxonal damage in patients with MS. These NAWM abnormalities have been observed in patients with RRMS but are more severe in those with progressive forms of the disease. They include decreased nerve fiber density resulting from axonal degeneration and demyelination, infiltration of small round cells (mainly lymphocytes) and macrophages, diffuse activation of microglia, and gliosis[39].

As mentioned earlier, while demyelinating lesions are more easily identifiable in the WM, their presence alone does not always explain or predict the clinical and radiological features seen in MS patients. This clinical-radiological paradox has been largely resolved by accumulating evidence from histopathological and high-resolution imaging studies, which have revealed that GM is also involved in the demyelination process, in the form of either focal GMLs or diffuse cortical atrophy.

GMLs can occur in different brain regions, including the cerebral cortex, thalamus, hippocampus, cerebellum, and spinal cord. Peterson and colleagues[29] have conducted studies on these lesions, noting axonal and dendritic transection, neuronal death by apoptosis, and reduced inflammatory cell content compared to WM lesions. Moreover, they described a significant association between microglia and neurons in cortical MS lesions[29].

Based on the pattern of cortical demyelination, they identified three different types of cortical lesions *(Figure 1):*

i) *Type I* are leukocortical lesions which involve both subcortical WM and cortex;

ii) *Type II* lesions reside entirely within the cortex, are usually small, and often contain a vessel at their center;

iii) *Type III* (the most frequent pattern) are subpial lesions that extend from the pial surface into the cortex; many include entire and commonly multiple gyri, extending to cortical layers 3 or 4.

When the classification proposed by Peterson was applied to biopsy samples from patients with early-stage MS, 38% showed clear evidence of cortical demyelination and a strong association with adjacent meningeal inflammation[40].

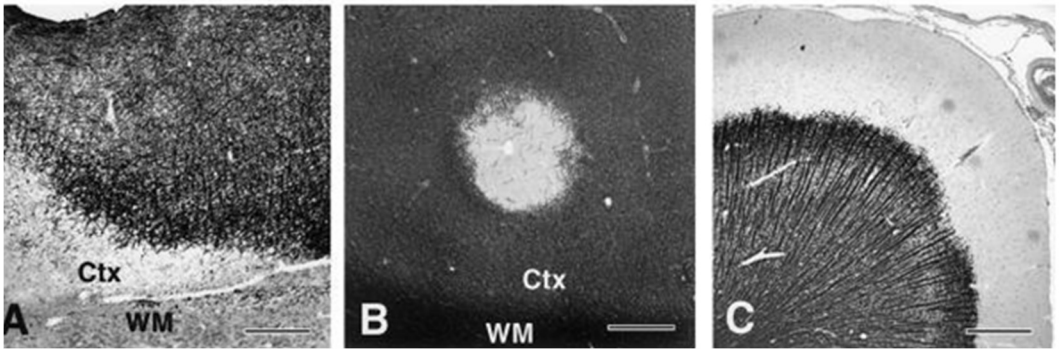


Figure 1. **Immunocytochemical distribution of myelin in the cerebral cortices of MS brains identified 3 patterns of demyelination.** (A) Type I lesions involved both subcortical WM and the cortex (Ctx). (B) Type II lesions were confined to the cortex and often contained a centrally located vessel. (C) Type III lesions extended from the pial surface into the cortex.

[Adapted from: Peterson JW, Bö L, Mörk S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical MS lesions. Ann Neurol. 2001;50(3):389-400. doi:10.1002/ana.1123].

After the first characterization of GMLs *ex vivo*, subsequent studies have shown that GM involvement can be detectable *in vivo* as well, even in the early stages of MS. This can be achieved by assessing cortical atrophy from conventional T1-weighted images[41] or by visualizing cortical lesions using specific GM imaging sequences such as Double Inversion Recovery (DIR)[42].

Interestingly, GMLs have been detected in some cases even before the appearance of WMLs, indicating that the development of cortical inflammation is at least partly independent of WM pathology[43].

GM involvement has shed light on the observed dissociation between markers of inflammatory demyelination (e.g., relapses, WM gadolinium enhancement, T2-

weighted lesion load) and disease progression. The rate of disability progression in both relapsing and progressive phases appears to be strongly linked to degenerative GM demyelination and diffuse cortical atrophy[44].

Based on these findings, it has been proposed that there are three fundamental pathologic processes in MS: the hallmark of acute or relapsing MS is represented by WM demyelination in the form of focal inflammatory lesions, whereas chronic progressive MS additionally includes diffuse damage in the NAWM and cortical demyelination[39]. These processes can occur in parallel as well as independently from each other, contributing to the heterogeneity and complexity of MS course.

1.1.1.4. Pathogenesis

Tissue damage in MS results from a complex and dynamic interplay between the immune system, glia (myelin-making oligodendrocytes and their precursors, microglia, and astrocytes), and neurons[35]. The traditional view of T cell-mediated MS relapses has evolved to include key bidirectional interactions between peripheral immune cells and CNS-resident cells. This cross-talk can result in the secretion of a range of neurotoxic mediators (e.g., cytokines, chemokines, and reactive oxygen species), which can promote and sustain inflammation, neuro-axonal damage, and neurodegeneration[45].

The historical view of MS, based on studies of patients and animal models with Experimental Autoimmune Encephalomyelitis (EAE), is that relapses are primarily mediated by aberrantly activated and/or insufficiently regulated pro-inflammatory CNS-specific effector T cells[1]. Indeed, both helper (CD4+) and cytotoxic (CD8+) T cells have been observed in MS lesions, with CD4+ cells more concentrated in the perivascular cuff and CD8+ cells widely distributed within the parenchyma[46].

The activation of T cells in MS requires antigen presentation by antigen-presenting cells (APCs), including B cells and myeloid cells (e.g., macrophages, dendritic cells, and microglia), both in the periphery and the CNS. However, the specific antigens responsible for this activation have not been conclusively identified[47]. Myelin-related antigens are the most suspected but some studies have also suggested antigens on the neuronal or glial cell surface. Noteworthy is that myelin-reactive T cells have been observed in similar proportions in individuals

with and without MS, suggesting either that these cells are dysfunctional in MS or that other immune factors also play critical roles[48].

How aberrantly activated immune cells access the CNS is of ongoing interest and therapeutic importance. Even though the CNS was traditionally considered immune privileged, with the blood-brain barrier (BBB) thought to restrict the entry of cells and macromolecules from the circulation, evidence of BBB breakdown has been observed in MS patients. This breakdown may facilitate the migration of pro-inflammatory cells into the CNS parenchyma. Also of note, in addition to the post-capillary venule BBB endothelial cells, which are the site of the classical perivascular MS lesions, immune cells might enter the CNS through other routes, such as the subarachnoid space and the blood-CSF barrier[49,50].

Due to the early and significant success of B-cell-depleting antibodies in limiting MS lesion formation and clinical disease activity, there is renewed attention on the role of B cells in MS[51,52]. It has long been recognized that the CSF of most people with MS contains unique antibodies known as “oligoclonal bands”, which are produced within the CNS. There is evidence that the antibody-producing function of B cells is important in some MS lesions[33]. However, considering the rapidity of the clinical response to B cell depletion, observed as early as 8-12 weeks, even before the reduction of circulating immunoglobulins, other antibody-independent functions of B cells are likely to be more relevant, including antigen presentation to helper T cells and cytokine production. MS relapses may also be driven by alterations in the balance between pro-inflammatory and anti-inflammatory B cells. This is supported by the observation that, apart from anti-CD20 therapies, other approved drugs for MS affect memory B cell responses. As further support, clinical trials have shown that Atacicept exacerbated MS relapses, indicating that selective loss of certain B cells subsets while sparing memory B cells can lead to a more pro-inflammatory B cell profile and worsen the disease[53].

Cells of the innate immune system are also important in MS pathogenesis[54]. Different populations of macrophages, including classically and alternatively activated macrophages, have been observed in active MS lesions, where they remove myelin debris and inflammatory byproducts. Microglia, the resident phagocytes of the CNS, are also abundant in MS lesions, but whether their role is

pathogenic or protective, or both, remains uncertain[55]. Microglial activation has been observed in the WM of MS autopsy specimens, even in areas remote from established lesions[56], and it may represent the earliest stage of lesion development, similar to what is observed in animal models[57]. Once activated, microglia and macrophages are pathologically indistinguishable, but recent progress using gene-expression technology has provided insights into their distinct contributions, potentially enabling the development of targeted therapies[58].

Furthermore, the biological mechanisms underlying remission in MS are not well understood but it is unlikely to be a mere passive decline in pro-inflammatory effector cell activity. Instead, it likely involves active processes that downregulate immune responses, such as the activity of regulatory T cells[59] and apoptosis of myelin-reactive T cells[60]. These mechanisms may contribute to the modulation of the immune system and the resolution of inflammation in MS.

1.1.1.5. Clinical presentation

The onset of MS can be abrupt or insidious. In the majority of cases (approximately 85%), patients seek medical attention because of an initial clinical attack, known as clinically isolated syndrome (CIS). CIS represents the first episode of neurological dysfunction with features suggestive of MS. To be termed CIS, the episode should last for at least 24 hours and not be attributed to fever or infection. In addition, there should be no clinical features of encephalopathy, such as altered consciousness or epileptic seizures[61]. The conversion rate from CIS to MS has been documented in previous studies to range from 30% to 82%[62–64]. The specific symptoms experienced by patients during CIS depend on the location and severity of demyelinating lesions within the CNS, typically involving the optic nerve, spinal cord, brainstem, cerebellum, or cerebral hemispheres *(Figure 2)*[1].

During the course of RRMS, further clinical episodes may take place, referred to as relapses. These exacerbations typically have an acute or sub-acute onset, gradually worsen over days or weeks, reach peak severity within 2-3 weeks, and then subside to varying degrees, ranging from minimal improvement to complete recovery[65].

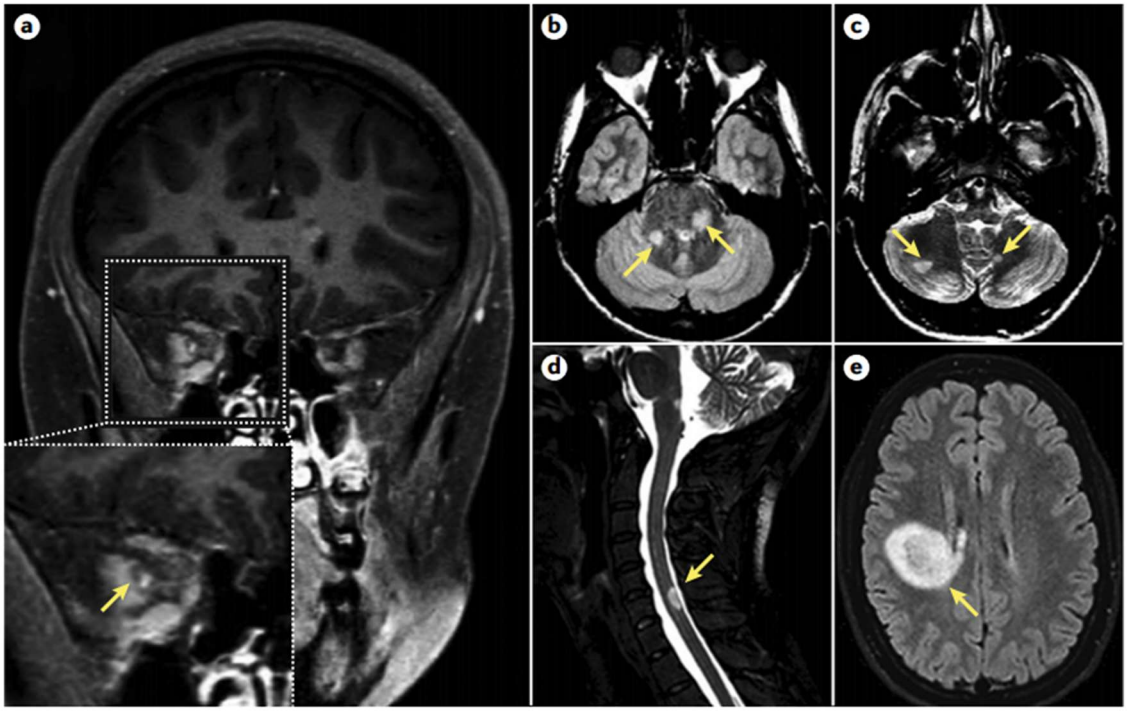


Figure 2. **3T MRI sequences from five patients with CIS suggestive of MS**, within 5 days from clinical onset, are shown. Focal lesions (arrows) can be observed in the right optic nerve in a patient with acute optic neuritis (a); the left pons and the right middle cerebellar peduncle in a patient with diplopia (b); the cerebellar hemispheres in a patient with vertigo (c); the cervical spinal cord in a patient with paresthesia and Lhermitte sign (d); and the left cerebral hemisphere in a patient with right sensorimotor hemisyndrome (e).

[Filippi M, Bar-Or A, Piehl F, Preziosa P, Solari A, Vukusic S, Rocca MA. Multiple sclerosis. Nat Rev Dis Primers. 2018 Nov 8;4(1):43. doi:10.1038/s41572-018-0041-4].

No clinical findings are unique to MS, but certain symptoms are highly characteristic of the disease. These include optic neuritis, bilateral internuclear ophthalmoplegia, spinal cord syndromes (resulting in sensory and/or motor symptoms), cerebellar ataxia, and vertigo[1].

Although it may seem straightforward at first glance, recognition of CIS symptoms in clinical practice can be challenging due to their subjective nature and the requirement to persist for at least 24 hours. In addition, symptoms like fatigue, cognitive dysfunction, sleep disorders, and affective disturbances are common in MS patients but cannot be classified as CIS or relapse of MS by themselves. However, the concept of cognitive relapse is gaining recognition and has been observed in clinical settings and trials[66]. Cognitive deficits may also predict conversion to clinically definite MS in patients with CIS[67].

Different scales have been suggested to assess the clinical manifestations of MS. Among them, the Expanded Disability Status Scale (EDSS, *Table 1*)[68] is the most widely used tool to measure the degree of disability in patients. It ranges from 0 (a completely normal neurological examination) to 10 (death due to MS). It provides eight subscales to evaluate the main functional systems affected by MS, including pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, mental, and other domains.

Table 1. The Expanded Disability Status Scale (EDSS).

0.0 = Normal neurologic examination (all grade 0 in functional systems [FS])
1.0 = No disability, minimal signs in one FS (i.e., grade 1)
1.5 = No disability, minimal signs in more than one FS (more than one grade 1)
2.0 = Minimal disability in one FS (one FS grade 2, others 0 or 1)
2.5 = Minimal disability in two FS (two FS grade 2, others 0 or 1)
3.0 = Moderate disability in one FS (one FS grade 3, others 0 or 1) or mild disability in three or four FS (three/four FS grade 2, others 0 or 1) although fully ambulatory
3.5 = Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2 (others 0 or 1)
4.0 = Ambulatory without aid or rest for 500m
4.5 = Ambulatory without aid or rest for 300m
5.0 = Ambulatory without aid or rest for 200m
5.5 = Ambulatory without aid or rest for 100m
6.0 = Unilateral assistance required to walk about 100m with or without resting
6.5 = Constant bilateral assistance required to walk about 20m without resting
7.0 = Unable to walk beyond about 5m even with aid; essentially restricted to wheelchair; wheels self and transfers alone
7.5 = Unable to take more than a few steps; restricted to wheelchair; may need aid to transfer
8.0 = Essentially restricted to bed or chair or perambulated in a wheelchair, but out of bed most of the day; retains many self-care functions; generally has effective use of arms
8.5 = Essentially restricted to bed much of the day; has some effective use of arms; retains some self-care functions
9.0 = Helpless bed patient; can communicate and eat
9.5 = Totally helpless bed patient; unable to communicate and eat
10.0 = Death to due MS

Source: Adapted from [Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). *Neurology*. 1983; doi:10.1212/WNL.33.11.1444]

1.1.1.6. Diagnosis

Diagnostic criteria for MS have evolved to allow earlier, more sensitive, and more specific diagnoses. The diagnosis of MS is based on three cornerstones:

i) *Dissemination in space (DIS):* it refers to the occurrence of lesions in distinct anatomical locations within the CNS, indicating a multifocal process;
ii) *Dissemination in time (DIT):* it involves the development of new lesions over time, indicating that the disease is not a result of a single event;
iii) "*No better explanation*": other possible differential diagnoses should be excluded to ensure that MS is the most likely explanation for the observed clinical, radiological, and laboratory findings.

The first diagnostic criteria were proposed in 1965 by Schumacher and were based on clinical assessment. In 1983, Poser revised the criteria, introducing the examination of the cerebrospinal fluid (CSF). After Barkhof's criteria (1997), the publication of McDonald criteria in 2001 introduced the neuro-radiological definitions of dissemination in time and space. Subsequent revisions by Polman's (2005) and McDonald's (2010) further refined the criteria. The McDonald criteria were finally modified in 2017 to simplify their use in clinical settings *(Figure 3)*.

In the 2017 revision, the following changes were made [69]:

i) In patients with a typically CIS and clinical or MRI demonstration of DIS, the presence of CSF-specific oligoclonal bands (OCBs) allows the diagnosis of MS. OCBs indicate the synthesis of intrathecal antibodies and are associated with a higher risk of a second attack;
ii) Symptomatic lesions can be used to demonstrate DIS or DIT in patients with supratentorial, infratentorial, or spinal cord syndromes. This simplifies the application of MRI criteria without sacrificing accuracy;
iii) Cortical lesions can be used to demonstrate DIS.

No. of clinical attacks	No. of MRI lesions with objective clinical evidence[a]	Additional data needed for diagnosis of multiple sclerosis
Relapsing-remitting multiple sclerosis		
≥2	≥2	None[b]
≥2	1[c]	None
≥2	1	DIS demonstrated by an additional clinical attack implicating a different CNS Site or by MRI
1	≥2	DIT demonstrated by additional clinical attack, MRI, or CSF-specific oligoclonal bands
1	1	DIS demonstrated by additional clinical attack implicating a different CNS site or by MRI *and* DIT demonstrated by an additional clinical attack or by MRI or demonstration of CSF-specific oligoclonal bands
Primary progressive multiple sclerosis		
Required: 1 year of disability progression (retrospectively or prospectively determined) independent of clinical relapse Plus 2 of the following: 1 or more T2-hyperintense lesions characteristic of multiple sclerosis in 1 or more of the following brain regions: periventricular, cortical or juxtacortical, or infratentorial; 2 or more T2-hyperintense lesions in the spinal cord; presence of CSF-specific oligoclonal bands		

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; DIS, dissemination in space; DIT, dissemination in time; MRI, magnetic resonance imaging.

[a] Adapted with permission from *Lancet Neurology*.[14]

[b] Although no MRI is required for the diagnosis, an MRI scan of the brain should be obtained in all patients with a suspected diagnosis of MS unless not possible.

[c] One lesion as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location.

Figure 3. **Revised 2017 McDonald Criteria for the diagnosis of RRMS and PPMS.**

[Thompson AJ, et al. Diagnosis of multiple sclerosis: 2017 revision of the McDonald criteria. Lancet Neurol.2018;17(2):162-173. doi:10.1016/S1474-4422(17)30470-2]

The inclusion of cortical lesions as part of the diagnostic criteria is relevant, as these lesions are specific for MS. However, a large proportion of cortical lesions go under detected on conventional MRI using standard field strength for several reasons, including their small size, the lower myelin content of the cortex compared to WM, resulting in less MRI contrast when demyelination occurs, and the partial volume effects from adjacent CSF and WM. Furthermore, most of the GMLs affect the outer layers of the cortex, which are in close contact with the sub-arachnoid space, resulting in susceptibility artifacts at the interface between the cortex and CSF. In addition, in contrast to WMLs, cortical GMLs show a lack of substantial focal infiltration of blood-derived leukocytes into the cortex, complement deposition, and BBB damage[44].

Several improvements have been achieved in the last decades, through the introduction of inversion recovery and the development of GM-specific pulse sequences such as double inversion recovery (DIR) and phase-sensitive inversion recovery (PSIR), and by moving to high field 3 T and ultra-high field 7 T MRI systems[70] *(Figure 4)*.

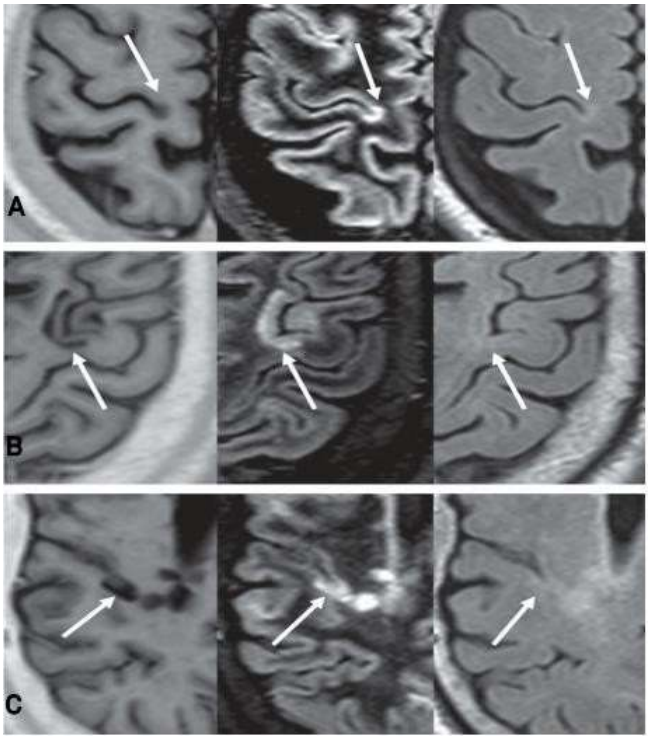


Figure 4. **Cortical lesions on PSIR** (left), **DIR** (center), **and FLAIR** (right). PSIR sequence is more sensitive in the detection of intracortical lesions (A), juxtacortical (B),and leukocortical (C).

[Nelson F, Poonawalla AH, Hou P, Huang F, Wolinsky JS, Narayana PA. Improved Identification of Intracortical Lesions in Multiple Sclerosis with Phase-Sensitive Inversion Recovery in Combination with Fast Double Inversion Recovery MR Imaging. *American Journal of Neuroradiology*. 2007;28(9):1645-1649. doi:10.3174/AJNR.A0645]

MS lesions have typical MRI signal and location characteristics, which aid in the diagnosis *(Figure 5)*. Lesions usually appear as multifocal, ovoid areas of increased signal on T2-weighted images. They are commonly found in the periventricular, juxtacortical, and infratentorial regions of the brain and the spinal cord. To distinguish active lesions from inactive ones, gadolinium-based contrast agents are administered, and post-contrast T1-weighted images are acquired.

Signal enhancement, which indicates active lesions, occurs due to increased BBB permeability and corresponds to areas with ongoing inflammation. Lesions that persistently appear hypointense on post-contrast T1-weighted images, known as *black-holes*, are associated with more severe tissue damage caused by long-lasting demyelination and axonal loss.

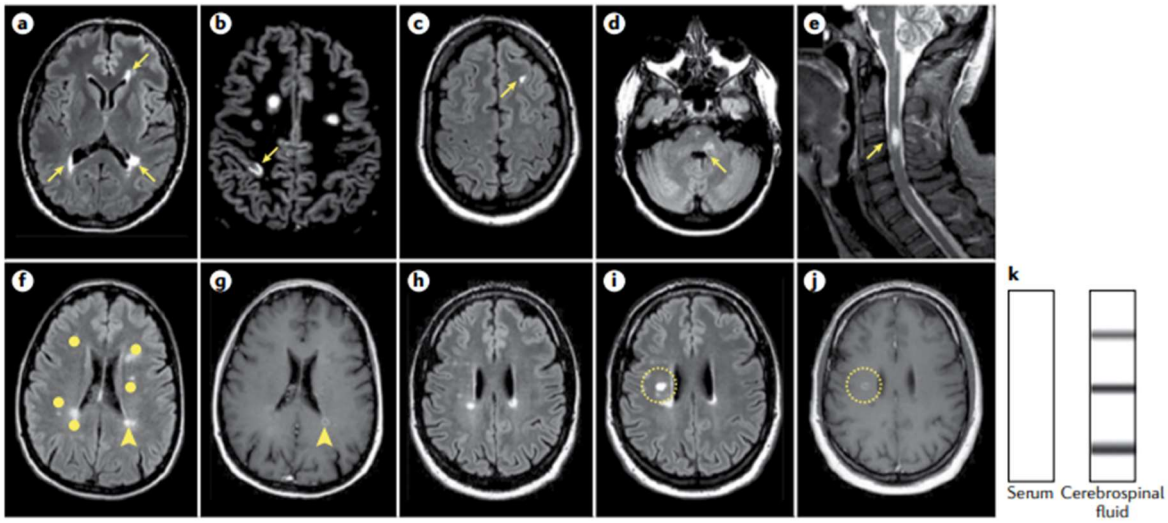


Figure 5. Demonstration of DIS and DIT in a patient with CIS suggestive of MS.
Parts a-e DIS can be demonstrated by ≥1 lesion in ≥2 of four typical areas of CNS (infratentorial, juxtacortical or cortical, periventricular, and spinal cord). These lesions can be identified through clinical events involving different areas of the CNS, multiple hyperintense lesions identified on MRI in T2 sequences or both. Specifically, WMLs appear hyperintense in T2 or FLAIR and iso/hypointense in T1, while the detection of GMLs can be improved with DIR sequences.
Parts f-k DIT can be demonstrated by a simultaneous presence of gadolinium-enhancing and non-enhancing lesions at any time and with the removal of the distinction between symptomatic and asymptomatic lesions, a new T2-hyperintense and/or gadolinium-enhancing lesion on follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI, or the presence of cerebrospinal fluid-specific oligoclonal bands, which are not visible in the serum.

[Filippi M, Bar-Or A, Piehl F, Preziosa P, Solari A, Vukusic S, Rocca MA. Multiple sclerosis. Nat Rev Dis Primers. 2018 Nov 8;4(1):43. doi:10.1038/s41572-018-0041-4].

The growing application of MRI has led to a significant increase in the detection of brain abnormalities suggestive of MS in asymptomatic individuals, a condition known as Radiologically Isolated Syndrome (RIS)[71]. Research indicates that up to 34% of RIS patients experience a clinical attack within 5 years. It has also been observed that male sex, younger age, and the presence of spinal cord lesions increase the risk of a first clinical event in RIS patients[72].

Aside from its use in the diagnosis, MRI has become essential in monitoring treatment efficacy and early recognition of treatment-related adverse effects, such as Progressive Multifocal Leukoencephalopathy (PML) caused by JC virus infection in patients using Natalizumab[73].

In addition to satisfying the aforementioned criteria, alternative diagnoses must be excluded. These include other inflammatory conditions of the CNS, such as Neuromyelitis Optica Spectrum Disorder (NMOSD), which is typically characterized by longitudinal spinal cord involvement beyond three segments, systemic inflammatory conditions (e.g., neurosarcoidosis), inherited disorders (e.g., Fabry disease), infections (e.g., neurosyphilis), toxic and nutritional disorders (e.g., B12 deficiency), tumors (e.g., glioblastoma), and vascular diseases (e.g., cerebral infarction). Acute Disseminated Encephalomyelitis (ADEM) is another important differential diagnosis, which is often associated with fever and preceded by exanthematous diseases, infections, or vaccinations[74].

1.1.1.7. Treatment

Remarkable progress has been made in the treatment of MS as a result of a deeper understanding of its pathogenetic mechanisms[75]. The development of highly effective disease-modifying therapies (DMTs) has resulted in nearly complete control of focal brain inflammation, reducing the frequency of clinical relapses and limiting the accumulation of WMLs on MRI[35] in RRMS patients.

However, currently available treatments poorly target CNS-compartmentalized inflammation, which is believed to contribute to CNS injury[76].

Moreover, effective treatment of progression remains an unmet need because existing therapies provide only partial protection against the neurodegenerative component of MS. Although the long-term course of the disease has undoubtedly improved in the treatment era, further studies are necessary to understand the underlying factors contributing to the “silent progression” of MS and identify new targets for future therapeutic agents[75] *(Figure 6).*

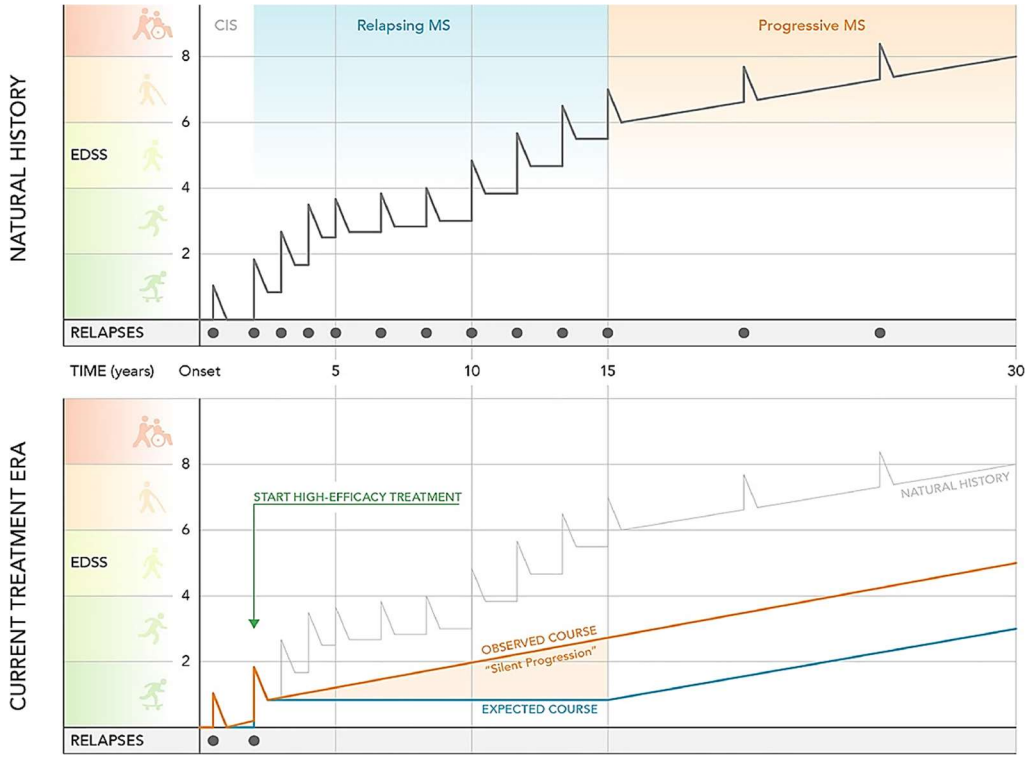


Figure 6. **The new natural history of MS.** The top half of the figure illustrates the natural history of RRMS. During the relapsing phase, disability progression was thought to result from incomplete recovery from relapses, until relapse-independent disability, designated as SPMS, supervened. The "new" natural history of MS in the treatment era is shown in the bottom half. With the use of highly effective therapies, attacks are abolished in most patients, but insidious progression independent of relapse activity, termed "silent progression", is now evident during the relapsing phase.

[Hauser SL, Cree BAC. Treatment of Multiple Sclerosis: A Review. Am J Med. 2020;133(12):1380-1390.e2. doi:10.1016/j.amjmed.2020.05.049].

Although MS has been categorized in distinct clinical forms (RRMS, SPMS, and PPMS) for patient care, research, and regulatory approval of medications, accumulating evidence suggests that the clinical course of MS should be viewed as a continuum, with contributions from concurrent pathophysiological processes that vary across patients and over time. The apparent transition to a progressive course reflects a shift from predominantly localized acute injury to widespread inflammation and neurodegeneration, accompanied by the failure of compensatory and reparative mechanisms, such as neuroplasticity and remyelination[77].

The understanding of the key mechanisms underlying progression and the introduction of new measures to quantify progressive pathology will potentially have important and beneficial implications for decision-making in clinical practice and research of new therapeutic targets[77].

1.2. Cognitive impairment in MS

"Conceptions are formed slowly and the intellectual and emotional faculties are blunted in their totality." (Charcot, 1877) [78]

Despite its precocious identification by Charcot, Cognitive Impairment (CI) has been overlooked for a long time in individuals with MS. However, in the past three decades, it has been increasingly investigated and it is now recognized to be a core and disabling feature of the MS clinical picture, with a negative influence on physical independence and competence in daily activities[79,80].

1.2.1. Prevalence of CI in MS

CI is highly prevalent in MS, affecting approximately 40-70% of patients, depending on the population studied and the neuropsychological assessment used[79,80]. Cognitive deficits can occur with any form of MS and at any disease stage, including early MS, even in the absence of other neurological deficits[79,80].

CI can progress insidiously and gradually over time, or abruptly during relapses. In recent years, the concept of isolated cognitive relapses (ICR) has emerged[66,80]. Pardini and colleagues[66] defined ICR as i) a transient significant cognitive decline in objective neuropsychological performance, ii) without clinical or subjective evidence of other new neurological signs and symptoms, and iii) associated with brain disease activity defined as a positive gadolinium-enhancing scan.

Overall, the frequency and severity of CI tend to increase over time and become more pronounced in the progressive forms of MS [79,80]. The prevalence of CI is approximately 20-25% in CIS and RIS, 30-45% in RRMS, and 50-75% in SPMS[79,80]. However, it has been demonstrated that the main factors associated with CI are greater physical disability, measured by EDSS, and older patients' age, rather than longer disease duration or the MS subtype per se[81].

Other factors may contribute to the variability in cognitive profiles among MS patients.These include genetic determinants, environmental factors, comorbidities, and the concept of cognitive reserve.

Cognitive reserve can be described as the individual resilience to brain damage and is measurable using the Cognitive Reserve Index, which takes into account factors such as education level, premorbid leisure activities, and IQ[82].

The presence of CI has been associated with a worse prognosis, including an increased risk of conversion from CIS to clinically definite MS[67], increased risk of disability progression over time[83], as well as increased risk of death[84]. Moreover, CI impacts participation in social activities, driving abilities, and employment status, reducing patients' overall quality of life. Therefore, given its clinical and prognostic relevance, routine assessment of CI is of critical importance for a comprehensive evaluation of MS patients[79,80].

1.2.2. Cognitive profiles in MS

Like all symptoms of MS, CI is characterized by high inter-patient variability, but some cognitive domains are more commonly affected than others, including cognitive processing speed (CPS), learning and memory, visuospatial abilities, and executive functions[80].

However, when assessing the pattern of cognitive deficits in individual patients, specific cognitive profiles can emerge. A recent study based on a large cohort of 1212 MS patients identified five phenotypes of cognitive functioning through latent profile analysis: preserved cognition (detected in 19.4% of subjects), mild verbal memory/semantic fluency involvement (29.9%), mild multidomain involvement (19.5%), severe-executive/attention involvement (13.8%), and severe multidomain impairment (17.5%)[85].

Beyond the aforementioned cognitive domains, other cognitive functions can be affected in MS. For instance, recent observations have reported significant impairment of the core aspects of social cognitive processing in MS patients[86]. Additionally, relatively less evaluated aspects such as altered emotion perception may also contribute to CI[87].

General intelligence and language, generally spared in adult-onset MS patients, can be impaired in pediatric-onset MS (POMS)[88], which manifests before the age of 18 years. During this critical developmental period, MS-related brain damage can interfere with normal neuronal maturation and the accrual of cognitive reserve, buffering the effects of demyelination and atrophy later in life[89]. CI is detectable in approximately one-third of people with POMS and can have a heterogeneous course over time, with an overall tendency toward recovery at group level[88]. However, a recent 12-year observational study revealed that the proportion of patients with impairment at the final evaluation was more than double that observed at baseline[90]. Additionally, worse cognitive performances have been associated with lower psychosocial attainment later in adulthood[91].

At the other end of the lifespan, late-onset MS (LOMS), which manifests after the age of 50, exhibits a peculiar neuropsychological profile. CI in LOMS poses diagnostic challenges as it needs to be differentiated from other causes of cognitive decline, such as Alzheimer's disease and vascular dementia[79,80].

Several confounders should be considered in the cognitive evaluation of MS patients. Among these, depression, anxiety, fatigue, and sleep disorders have been studied more extensively[79,80]. For instance, depression could negatively affect working memory and executive control specifically[92]. Likewise, fatigue and sleep disorders have been linked to deficits in processing speed, memory, attention, and executive functions[93,94]. Primary failure of key brain regions involved in emotional processing or abnormal connectivity between them can lead to the adoption of maladaptive cognitive strategies and the development of mood disorders[95].

1.2.3. Pathogenesis of CI

The precise mechanisms underlying CI in MS are still largely unknown.

MRI studies have shown that focal brain inflammatory lesions may play a key role in the interruption of neuronal pathways involved in cognitive functioning[27]. This evidence has led to the hypothesis that MS-related CI arises from a 'disconnection syndrome', primarily affecting brain WM[96]. However, although many studies have described associations between brain lesion load on T2-

weighted images and neuropsychological performance[27], the origin of CI in MS appears more complex and multifactorial, involving both WM and GM pathology. Recently, MRI studies using DIR sequences have provided evidence that focal cortical lesions are associated with CI[97,98]. However, as observed for focal WM damage, cortical lesions alone are not sufficient to explain MS-related CI[99].

Among focal lesions, a potential role in cognitive dysfunction has been hypothesized for areas of active brain inflammation, indicated by the presence of contrast-enhancing lesions on MRI[100]. These lesions may cause transient cognitive deficits because of the detrimental effect of focal demyelination on circuits' dynamics[66]. However, this evidence also suggests that neuronal network functioning is influenced not only by the 'disconnecting' effect of focal WMLs but also by the more diffuse effects of immune molecules that are released during inflammation and/or BBB breakdown[101]. In this context, it should be acknowledged that other studies have yielded conflicting results, suggesting that MRI markers of inflammation are not associated with neuropsychological performance in MS and that neurodegeneration is the major determinant of patients' cognitive dysfunction[102].

Neurodegeneration certainly plays a major role in the pathogenesis of cognitive dysfunction in MS. Cognitive performance has been linked to the presence of diffuse cortical atrophy[97,103,104] and atrophy of key GM neuronal structures during MS, such as the thalamus[105], putamen[106], hippocampus[107], cerebellum[108], corpus callosum[109] and the amygdala[110]. Thus, beyond focal damage, an important role in the pathogenesis of MS-related CI is played by NAWM and NAGM, whose involvement has been demonstrated by volumetric, metabolic, and microstructural MRI studies[27].

In addition to structural analysis, studies have increasingly focused on the functional connectivity of GM structures by means of resting-state functional MRI (rs-fMRI). These studies have noted altered connectivity patterns in MS patients with CI[111,112]. However, the direction of the relationship between functional connectivity and CI is inconsistent, with some studies reporting increased[113] and others decreased connectivity [114] between cognitive-related regions.

Different hypotheses can be formulated to explain these findings. In the early stages, increased connectivity may signify that neuronal resources are compensating for demyelination and neuronal loss, so cognitive changes are subtle or not visible. This may be an adaptive mechanism to neuronal injury, with increased cortical recruitment of cognitive-related areas. As the disease progresses, these reserve resources are exhausted, the compensation is lost, and connectivity diminishes, leading to the manifestations of CI. Overall, these fMRI studies indicate that cognitive decline is explained by an accruing destabilization of the brain network physiology[115].

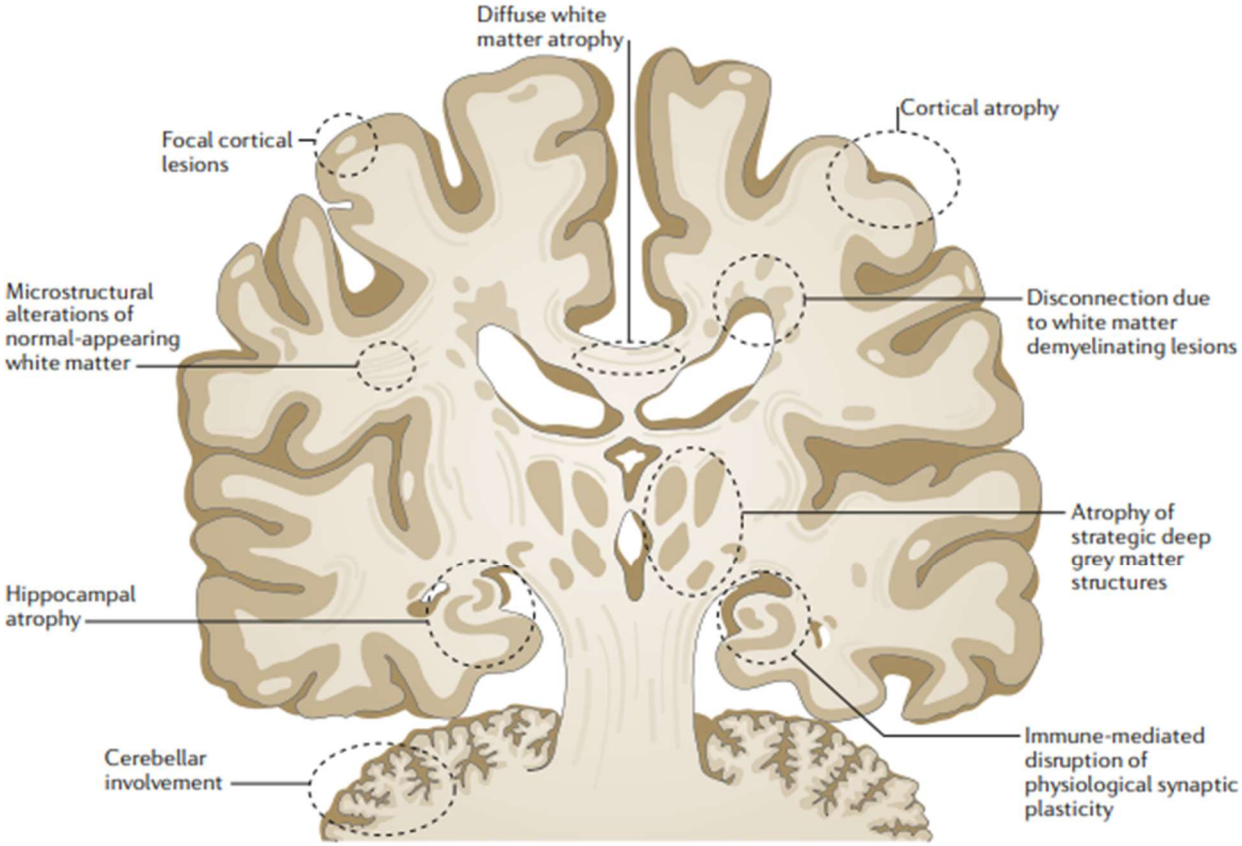


Figure 7. Putative mechanisms underlying CI in MS. In WM, focal demyelinating lesions, microstructural alterations in NAWM, and diffuse atrophy may interrupt neuronal pathways between brain areas strategic for physiological cognitive functioning, leading to a 'disconnection syndrome'. At the same time, a role is played by diffuse and focal cortical pathological changes and dysfunction in specific structures, such as deep GM nuclei (including the thalamus), the hippocampus, and the cerebellum. A pathogenic role may also be played by immune-related disruption of physiological synaptic plasticity.

[Di Filippo M, Portaccio E, Mancini A, Calabresi P. Multiple sclerosis and cognition: synaptic failure and network dysfunction. Nat Rev Neurosci. 2018;19(10):599-609. doi:10.1038/s41583-018-0053-9]

In a more integrated view, all these mechanisms synergistically contribute to the disruption of structural and functional connections that are fundamental for normal network functioning. Damage to brain regions (i.e., the nodes of the network), as well as their anatomical and functional connections (i.e., the edges of the network), progressively reduces network efficiency until the network collapses, which leads to faster neurodegeneration and accelerated clinical and cognitive deterioration[116].

1.2.4. Neuropsychological assessment

CI is often under-recognized and under-treated in MS patients because it is rarely evaluated in routine clinical practice. Systematic neuropsychological assessment from the beginning of MS and throughout the disease course may allow early recognition of CI. This approach allows for the implementation of management strategies at a younger age, when the patient's compensatory abilities, brain plasticity, and cognitive reserve may better mitigate the effects of CI before it becomes an established and irreversible entity[117].

Cognition in MS may be assessed by two complementary modalities: i) the self-reported evaluation of MS patients and their relatives, and ii) the neurocognitive batteries specifically adapted for the disease[118].

The self-reported CI method has its limitations as cognitive symptoms are often hidden by more visible deficits (e.g., motor, sensory, cerebellar) and can be influenced by depression, fatigue, or medication side effects. Patients themselves may not fully recognize their cognitive difficulties[118]. In contrast, evaluations from relatives and caregivers tend to be more reliable. Even so, self-perceived CI is important for patients to be aware of its impact on their daily activities[119].

The selection of neuropsychological tests for the assessment of MS-related CI is still a matter of debate in the literature. The convention in neuropsychology is to define CI when performance falls more than 1.5 SD below the expected normal values, after accounting for demographic factors such as age and education[80]. Ideally, these tests should be sensitive, reproducible, reliable, easy to administer, and time-efficient, and they should also consider the patients' comfort, available resources in MS clinics, and implied costs[80].

Since the seminal work by Rao and colleagues[120], different cognitive batteries specific to MS have been developed, mainly focusing on domains known to be affected by the disease. Consequently, tests assessing processing speed, attention, learning and memory have been more frequently included.

The Symbol Digit Modalities Test (SDMT) has demonstrated higher sensitivity in detecting cognitive dysfunction in MS and is now widely acknowledged as the gold standard for quick cognitive screening[121]. However, this test is not specific to MS and only evaluates processing speed, overlooking other relevant cognitive domains such as learning and memory. Therefore, a more comprehensive assessment should always be considered in cases of failure in cognitive screening or cases of high suspicion of CI and normal performance on the screening tools.

Table 2 provides an overview of the main batteries used to assess CI in MS.

Table 2. The main validated batteries to assess CI in MS patients.

	Duration of Administration	Tests Included	Cognitive Functions Assessed
Screening tests			
Symbol Digit Modalities Test (SDMT)	5 min	Symbol Digit Modalities Test (SDMT)	Information processing speed
Processing Speed Test (PST)	5 min	Processing Speed Test (PST)	Information processing speed
Computerized Speed Cognitive Test (CSCT)	5 min	Computerized Speed Cognitive Test (CSCT)	Information processing speed
Brief Neuropsychological Batteries			
Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS)	15 min	Symbol Digit Modalities Test (SDMT) California Verbal Learning Test–2nd ed. (CVLT-II) Brief Visuospatial Memory Test–Revised (BVMTR)	Information processing speed Verbal learning and memory Visuospatial learning and memory
Brief Repeatable Neuropsychological Batter (BRNB)	45 min	Paced Auditory Serial Addition Test (PASAT) Symbol Digit Modalities Test (SDMT) Selective Reminding Test (SRT) 10/36 Spatial Recall Test (SPART) Controlled Oral Word Association Test (COWAT)	Attention Information processing speed Verbal learning and memory Visuospatial learning and memory Verbal fluency
Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS)	90 min	Paced Auditory Serial Addition Test (PASAT) Symbol Digit Modalities Test (SDMT) California Verbal Learning Test–2nd ed. (CVLT-II) Brief Visuospatial Memory Test–Revised (BVMTR) Controlled Oral Word Association Test (COWAT) Judgement of Line Orientation Test (JLoT) Delis-Kaplan Executive Function System Sorting Test (D-KEFS-ST)	Attention Information processing speed Verbal learning and memory Visuospatial learning and memory Verbal fluency Visuospatial perception Executive functions

[Portaccio E;, Amato MP, Portaccio E, Amato MP. Cognitive Impairment in Multiple Sclerosis: An Update on Assessment and Management. NeuroSci 2022, Vol 3, Pages 667-676. 2022;3(4):667-676. doi:10.3390/NEUROSCI3040048]

Two cognitive batteries that have been validated for the assessment of MS-related CI are the Brief Repeatable Battery of Neuropsychological tests (BRBN)[120] and the Minimal Assessment of Cognitive Function in MS (MACFIMS)[122]. They are comparable in their discriminative validity, with equal abilities to distinguish MS patients from healthy controls. However, because both are time-consuming and require special materials and experienced neuropsychologists to administer and interpret them, they are not commonly used in routine clinical practice[80].

In an effort to develop a more feasible screening tool during the clinical assessment of MS patients, a committee of neurologists and neuropsychologists created the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS)[123]. It takes 15 min to administer and does not require special materials. It assesses three cognitive domains *(Figure 8):*

i) *Cognitive Processing Speed (CPS)*, using the Symbol Digit Modalities Test (SDMT). The patient is asked to voice the digit associated with each symbol according to a given key as quickly as possible. The dependent variable is the total number of correct responses in 90 seconds;

ii) *Verbal learning and memory*, using the California Verbal Learning Test (CVLT-II). The examiner reads a list of 16 words, and the patient is asked to recall as many words as possible. The dependent variable is the total number of words recalled over five learning trials;

iii) *Visuospatial learning and memory*, using the Brief Verbal Memory Test-Revised (BVMT-R). The patient looks at a matrix of six abstract designs for 10 seconds. Then is asked to reproduce the designs using paper and pencil, taking as much time as needed. Each design receives a score of 0, 1, or 2 based on accuracy and location criteria. The dependent variable is the total recall score across three trials.

These tests were chosen with consideration of both their psychometric standards (reliability, validity, and sensitivity in MS) and practical standards (ease of administration, feasibility, and acceptability to patients). BICAMS is not designed to be a full cognitive assessment, but rather an accurate monitoring or screening instrument, for use in clinical settings. It has been validated as an accurate cognitive battery in more than 28 countries[117].

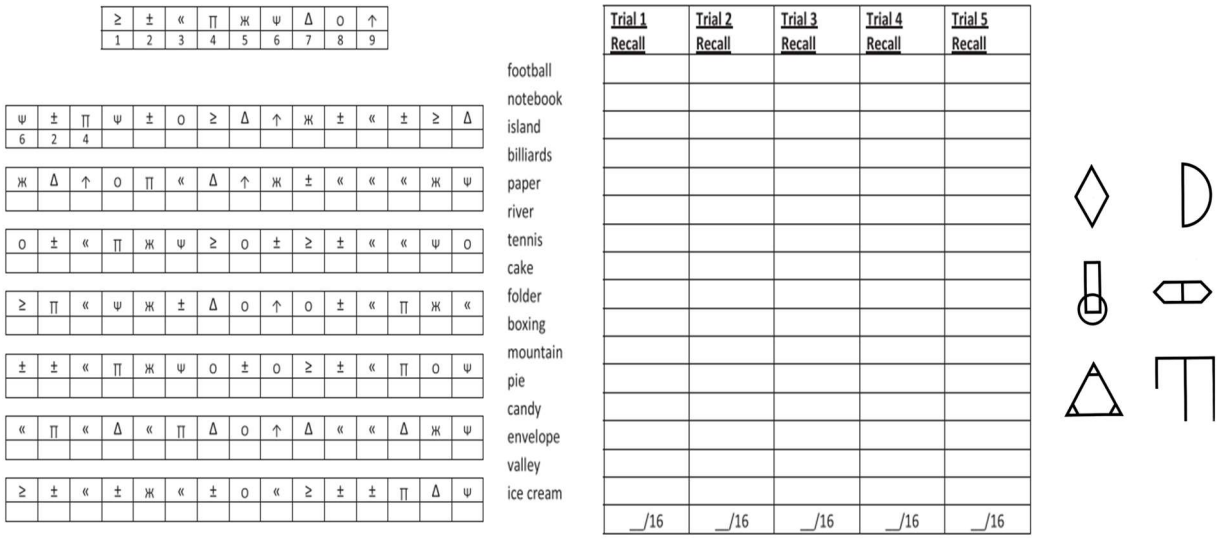


Figure 8. Facsimile of the three tests included in BICAMS. From left to right: the SDMT, the CLVT-II, and the BVMT-R.

[Langdon DW, Amato MP, Boringa J, et al. Recommendations for a Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS). Mult Scler. 2012;18(6):891-898].

Computerized testing can represent a valid alternative to conventional paper-and-pencil assessment to address time constraints and increase the feasibility of routine cognitive screening. Moreover, the automation of computer-mediated tests can minimize the need for trained professionals. To date, the validity of some computer-assisted tests has been established in patients MS[124], such as the Processing Speed Test (PST)[125], a tablet-based test modeled after the SDMT.

According to recent recommendations, cognitive screening should be performed during the initial evaluation of MS patients and repeated annually using the same instrument or more often if needed. In cases of cognitive failure at screening evaluation, a more comprehensive assessment should be planned. For children, cognitive evaluation should also include monitoring of academic and behavioural school performance[126]. Since mood, fatigue, and sleep disorders are widely acknowledged as important contributors to CI in MS, a comprehensive neuropsychological assessment should always include routine monitoring and screening of these factors to understand the patient's psychological state and difficulties. Recently, the Beck Depression Inventory [127], the Hospital Anxiety and Depression Scale[128], and the Fatigue Severity Scale[129] have been recommended for this purpose.

1.2.5. Treatment strategies

With the advent of DMTs for MS and the emphasis on early treatment, early detection of CI becomes crucial so that patients may benefit from symptomatic and rehabilitation interventions[118].

Currently, neuropsychological rehabilitation is the mainstay for the treatment of CI in MS. It aims to increase the patient's awareness of their own CI and develop strategies to cope with CI in daily life[117]. There are two main approaches[130]:

i) *Restorative cognitive rehabilitation* aims to reinstate cognitive skills, typically through repetitive cognitive exercises using computer-assisted paradigms. Among the available computerized programs, RehaCom has been extensively used in MS and has shown improvements in attention, information processing speed, memory, and executive functions[131];

ii) *Compensatory cognitive rehabilitation* does not aim to restore lost cognitive skills. Instead, it helps patients compensate for their cognitive difficulties by using various internal strategies (e.g., visualization) and external strategies (e.g., reminders). The modified Story Memory Technique has demonstrated valid for memory rehabilitation by training patients to use context and imagery as strategies to enhance the acquisition and retention of information[132].

Currently, there are no approved medications specific for MS-related CI. Treatment with DMTs is naturally expected to bring some benefits in cognitive functioning, together with the improvement in clinical outcomes and MRI parameters. However, there is limited evidence in the literature about their effect on cognition[79,80]. A recent systematic review and meta-analysis found a small to medium beneficial effect of DMTs on cognition, although the quality of the research was low for most of the studies[133]. In a subsequent systematic review, the authors did not find any significant effect[134]. Noteworthy is that cognitive outcomes have been included in phase 3 trials only in recent years [135,136], so higher-quality randomized controlled trials with cognitive parameters as primary endpoint are needed to establish the efficacy of DMTs in addressing MS-related CI[137].

There have been attempts to use licensed drugs for dementia diseases, such as acetylcholinesterase inhibitors (e.g., donepezil, rivastigmine, and galantamine)[138] and antagonist of NMDA receptors (e.g., memantine)[139]. However, the evidence supporting their efficacy in MS is insufficient, and their role in cognitive decline of MS patients remains controversial[79]. Some studies have shown the positive effects of dalfampridine (a potassium channel blocker) on cognitive measures, but further confirmation is required[140].

In addition to interventions targeting cognitive deficits, comprehensive management of neuropsychological dysfunction in people with MS should address mood disorders and other factors that impact cognition. Psychological interventions, including mindfulness-based approaches, have shown effectiveness in improving cognitive functioning in MS patients[141,142].

1.3. The Cerebellum's role in Cognition

Historically, the cerebellum's role in cognition has been a topic of debate[143].

Most people in the field of neurology have been taught that the cerebellum primarily functions as a co-processor of movement in concert with the cortex and basal ganglia. However, the past three decades have witnessed increasing evidence that the role of the cerebellum extends considerably beyond motor control. This conclusion is supported by the observation that there are i) anatomic connections between the neocerebellum and cognitive areas of the cerebral cortex, ii) neuroimaging studies showing cerebellar activation during a range of cognitive tasks, iii) phylogenetic findings of parallel expansion of neocerebellum and associative prefrontal cortical areas during human evolution, and iv) clinical populations in whom cerebellar damage produces non-motor deficits in cognition and behaviour, referred to as "Cerebellar Cognitive Affective Syndrome" (CCAS)[143]. A core tenet of this new understanding is the existence of a precise functional topography within the human cerebellum that differentially supports motor, cognitive, and affective behaviors[144].

1.3.1. Structural evidence of a cerebellar role in cognition

"In biology, if seeking to understand function, it is usually a good idea to study structure."[145]

The cerebellum consists of two cortex-covered hemispheres on either side of the midline vermis and connects to the brainstem via three paired cerebellar peduncles. The superior cerebellar peduncle carries fiber tracts that transmit information from the cerebellum to the cerebral cortex. Conversely, cerebral cortical projections to the cerebellum travel via the pontine nuclei and the middle cerebellar peduncle. The inferior cerebellar peduncle carries inputs to the cerebellum from the inferior olive, spinal cord, and vestibular system, as well as efferent fiber tracts from the cerebellum to the spinal cord[144,146].

Along the anterior-posterior axis, the cerebellum is divided into ten lobules that are grouped into three larger lobes. Lobules I–V form the anterior lobe, lobules VI–IX the posterior lobe, and lobule X the flocculonodular lobe[144,146] *(Figure 9)*.

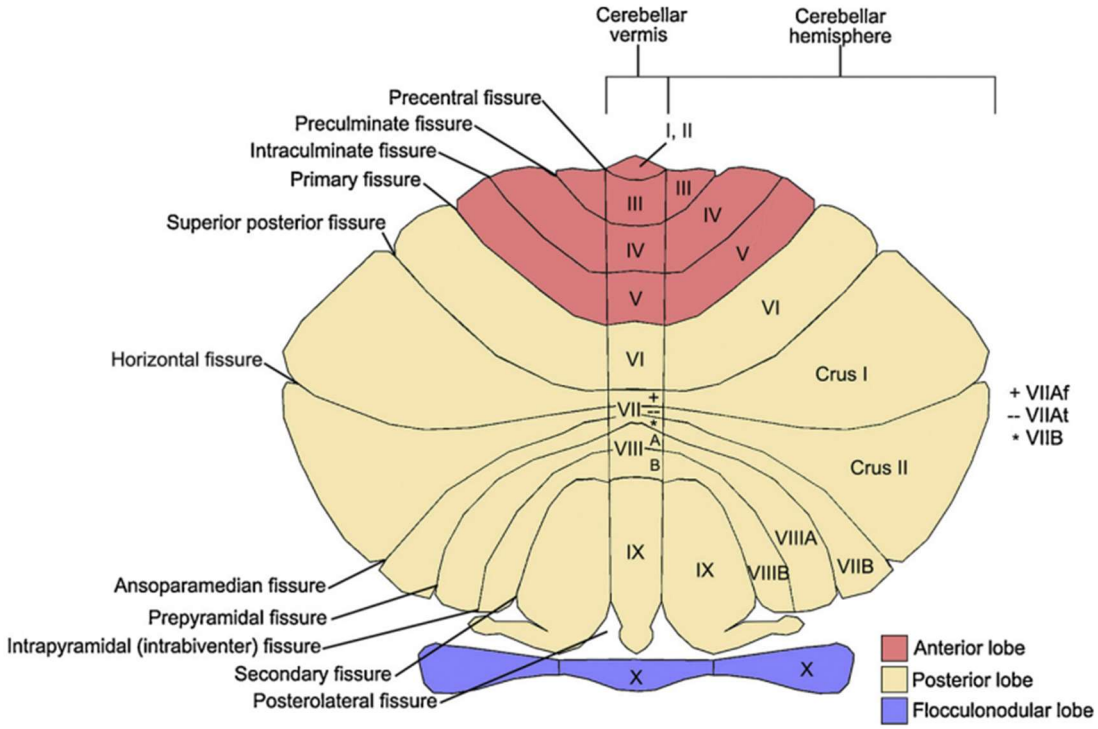


Figure 9. Flattened representation of the cerebellum and its major fissures, lobes, and lobules. The anterior lobe (lobules I-V) is shaded red; the posterior lobe (lobules VI-IX) is cream, and the flocculonodular lobe (lobule X) is purple. Lobules I through X are identified in the vermis and the hemispheres. In lobule VII, VIIAf at the vermis expands in the hemisphere to become Crus I, VIIAt at the vermis merges with Crus II in the hemisphere, and VIIB retains its designation both at the vermis and the hemispheres.

[Schmahmann JD. The cerebellum and cognition. *Neurosci Lett*. 2019;688:62-75. doi:10.1016/J.NEULET.2018.07.005]

Embedded in the WM of the cerebellum are the deep cerebellar nuclei, including the fastigial, interpositus (globose and emboliform), and dentate nuclei. The cerebellar cortex projects to the deep nuclei in a systematic medial-to-lateral pattern: the midline vermis projects to the medial fastigial nuclei, the paravermal regions to the interpositus nuclei, and the lateral hemispheres to the dentate nuclei. From the deep nuclei, projections travel through the superior cerebellar peduncle, passing through the controlateral red nucleus to the thalamus, where they synapse before being transmitted to the cerebral cortex, and through the inferior cerebellar peduncle to the brainstem nuclei and subsequently to the spinal cord. Lobule X projects directly to the vestibular nuclei[146,147].

According to Schmahmann's model, [148] the cerebellum can be divided into two functionally distinct parts: the "motor cerebellum" and the "cognitive cerebellum". On one hand, the motor cerebellum comprises the anterior lobe (lobules I to V), adjacent parts of lobule VI, and lobule VIII. It is primarily involved in sensorimotor processing, contributing to motor coordination, movement planning, and execution. On the other hand, the cognitive cerebellum consists of the remaining posterior lobe (lobules VII, adjacent parts of lobule VI, and lobule IX) and engages with higher cognitive circuitry, contributing to cognitive functions such as attention, language processing, working memory, and executive functions. Lobule X, known as the vestibulocerebellum, primarily attends to balance and vestibular reflexes and can, in a way, be considered part of the motor cerebellum due to its involvement in sensorimotor functions[148].

A representation of this model is shown in *Figure 10*.

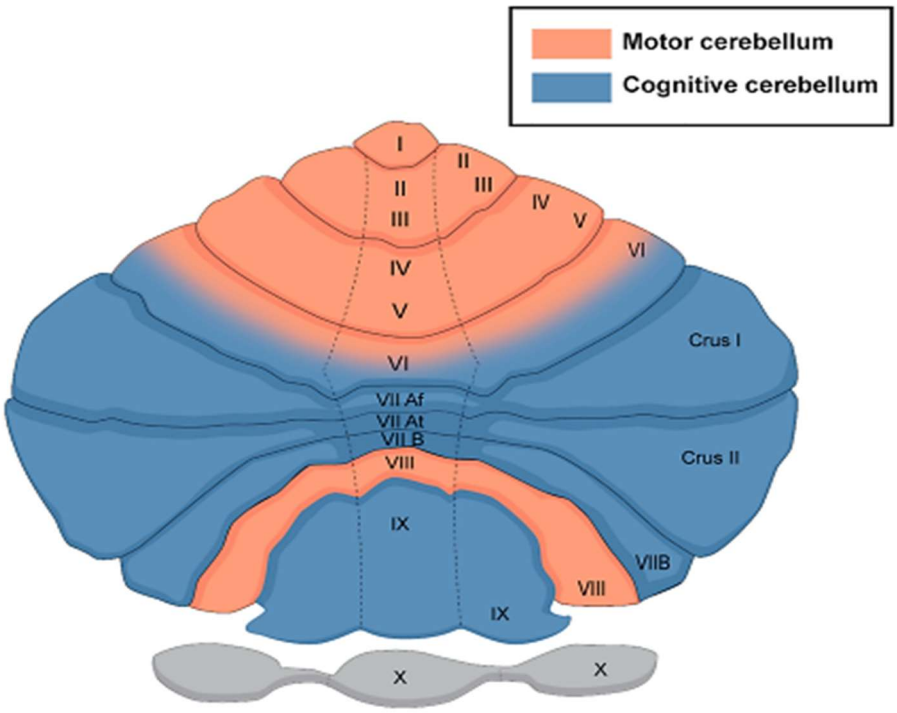


Figure 10. Unfolded view of the cerebellar cortex showing the functional organization of the cerebellum. In red the "sensorimotor" and in blue the "cognitive" cerebellum.

[Devita M, et al. Novel insights into the relationship between cerebellum and dementia: A narrative review as a toolkit for clinicians. Ageing Res Rev. 2021;70:101389]

The functional segregation of the cerebellum into motor and cognitive regions is also supported by its patterns of input and output connectivity, as demonstrated by neuroanatomical tract-tracing studies [149–151]. The cerebellum projects to precise cerebral, brainstem, and spinal cord destinations, and receives input back from these same regions, thus forming multiple, reciprocal circuits, or close loops. For instance, the cerebellum is reciprocally connected with the cerebral cortex via two-stage feedforward corticopontocerebellar loops and two-stage feedback cerebellothalamocortical loops[148]. The organization of these anatomic connections forms the basis of cerebellar functional topography (*Figure 11*) [149–151].

The motor cerebellum receives cutaneous-kinesthetic projections from the trigeminal nerve, spine, and medial (MAO) and dorsal (DAO) accessory olivary nuclei, which relay spinal and vestibular somatotopic information. Additionally, the anterior lobe receives input from primary and supplementary sensory and motor cortices via projections from the caudal pontine nuclei.

In contrast, the afferent pathways to the cognitive cerebellum are not involved in the sensorimotor circuitry. Lobule VII (which comprises ≈48% of the cerebellar cortex in humans[152], and is subdivided into crus I, crus II, and VIIB[153]) receives projections from the principal olivary nucleus (PO) which has no spinal input and is targeted by cortical projections. It also receives input from cerebral multimodal association areas relayed by the rostral pontine nuclei which are not as involved in the sensorimotor circuitry as the more caudal ones[148,154]. Specifically, relevant connections have been described with high-order cerebral association areas, including the posterior parietal cortices involved in spatial awareness, supramodal areas of the superior temporal gyrus important for language, posterior parahippocampal areas concerned with spatial memory, visual association areas in the parastriate cortices relevant for high-order visual processing, and multiple areas in the prefrontal cortex critical for complex reasoning, judgment, attention, and working memory[155].

Likewise, the cerebellar output pathways maintain the segregation between motor and non-motor systems. The motor cerebellum sends feedback motor projections to the spine, brainstem (MAO, DAO, and vestibular nuclei), ventrolateral thalamic nucleus, and cerebral motor cortices through the fastigial,

interpositus, and dorsal dentate nuclei. Conversely, the cognitive cerebellum sends efferents to the ventral dentate nucleus which, in turn, projects back to the PO and to non-motor thalamic nuclei (e.g., intralaminar) to close the loop with cerebral association cortices [148,154].

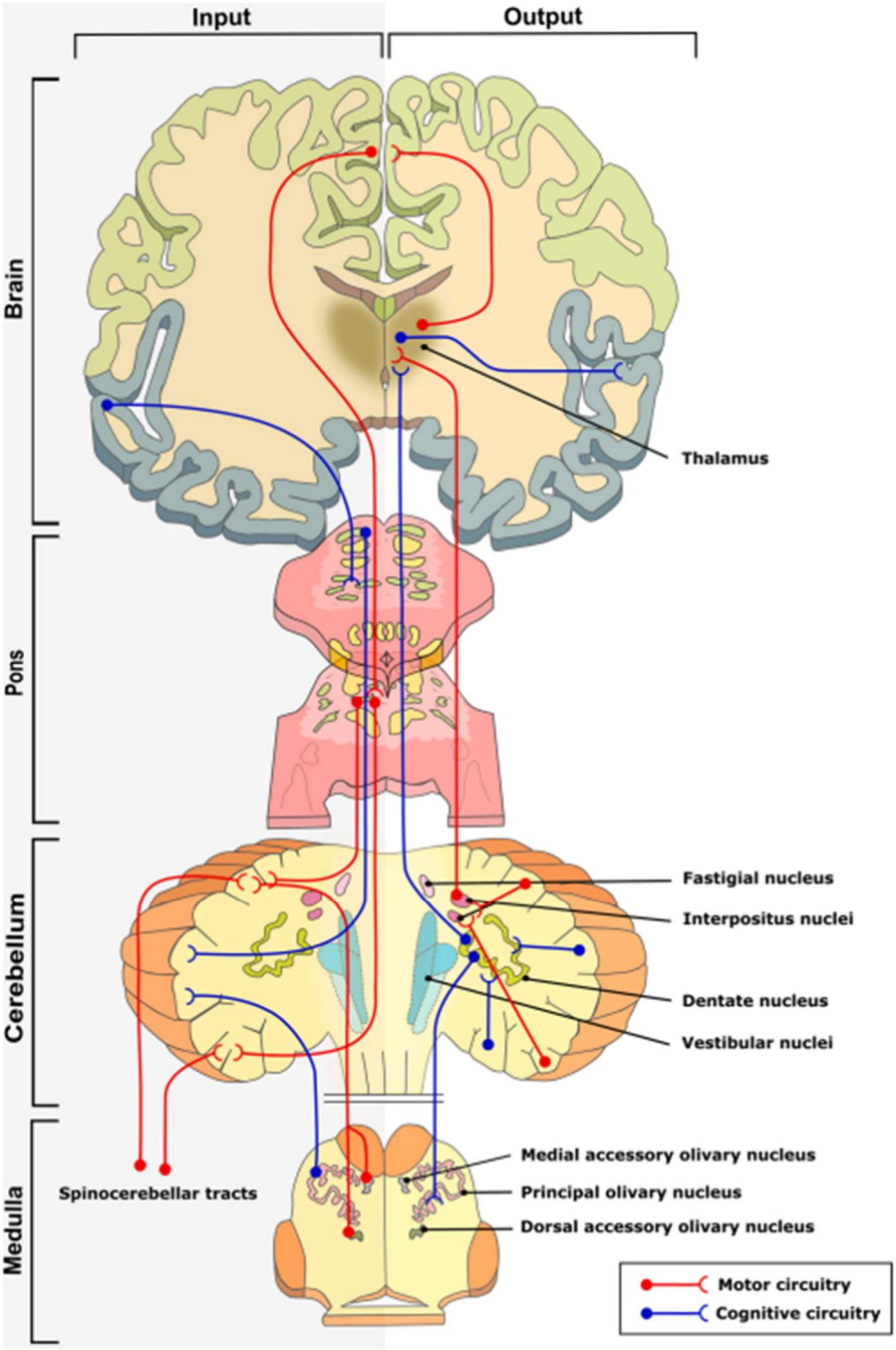


Figure 11. A representation of motor and non-motor connectivity of the cerebellum.

[Devita M, et al. Novel insights into the relationship between cerebellum and dementia: A narrative review as a toolkit for clinicians. Ageing Res Rev. 2021;70:101389]

The anatomical connections set up a dichotomy of cerebellar linkage with the cerebral cortex, brainstem, and spinal cord, and portray a complex connectivity and functional heterogeneity. The motor cerebellum appears to be an integral part of the distributed neural circuits responsible for motor functions, whereas the cognitive cerebellum is incorporated into the distributed neural circuits necessary for cognitive processing and emotion regulation[148].

1.3.2. Functional evidence of a cerebellar role in cognition

Looking beyond anatomy, a plethora of functional neuroimaging studies have provided evidence for the involvement of the cerebellum in cognition.

However, studying the cerebellum using MRI presents significant technical challenges. The cerebellar cortical GM is tightly folded and contains thin layers of WM, making it difficult to capture detailed imaging. Additionally, its location in the posterior fossa, surrounded by bone and vascular structures, further complicates MRI studies of this region. Only recently the difficulties concerning the segmentation of the thin cerebellar gyri and sulci and the extraction of the cerebellar tissue from nearby structures have been partially overcome[156].

MRI has emerged as a powerful tool for mapping the connectivity of the human brain, including cerebro-cerebellar circuits, providing new insights into the organization and functional connections of the cerebellum. Different forms of connectivity can be investigated using MRI *(Figure 12)*:

i) *Anatomical or structural connectivity* is usually acquired by diffusion tensor imaging (DTI), a technique that measures the diffusion of water molecules along WM tracts. DTI gives direct non-invasive information about the location and integrity of these tracts, allowing researchers to map the anatomical connections between different brain regions[157;]

ii) *Functional connectivity*, a term coined by Karl Friston (1994), refers to the statistical temporal dependency between the neural activity of spatially separated brain regions. It is typically estimated from functional MRI (fMRI)[158,159].

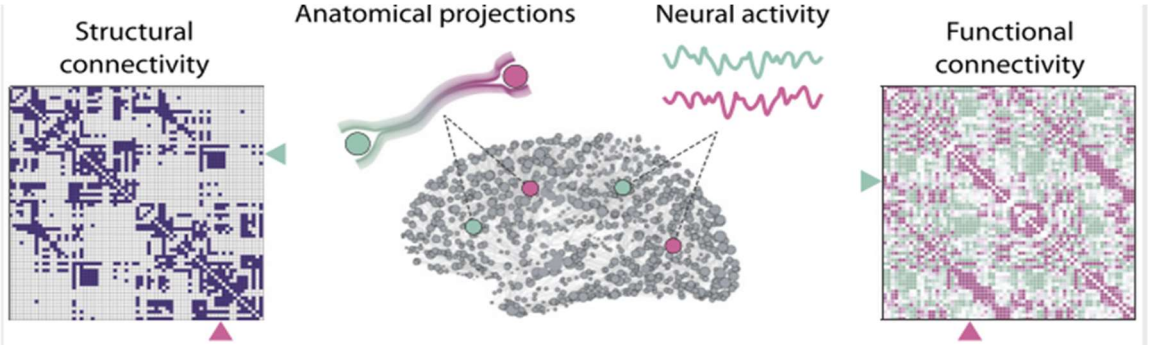


Figure 12. Measuring Structural and Functional Connectivity. At the macroscale level, structural and functional networks are derived by first parcellating the brain into GM nodes. For structural connectivity networks, edges are defined by reconstructing WM projections between network nodes. For functional networks, edges are defined by estimating statistical associations between node time courses.

[Suárez LE, Markello RD, Betzel RF, Misic B. Linking Structure and Function in Macroscale Brain Networks. *Trends Cogn Sci*. 2020;24(4):302-315. doi:10.1016/j.tics.2020.01.008]

fMRI detects changes in blood oxygenation level-dependent (BOLD) contrast, which indirectly reflects neural activity in the brain. It is based on the principle of neurovascular coupling: when a specific brain region becomes more active, it requires more oxygen and nutrients, leading to increased blood flow to that area *(Figure 13)*[160].

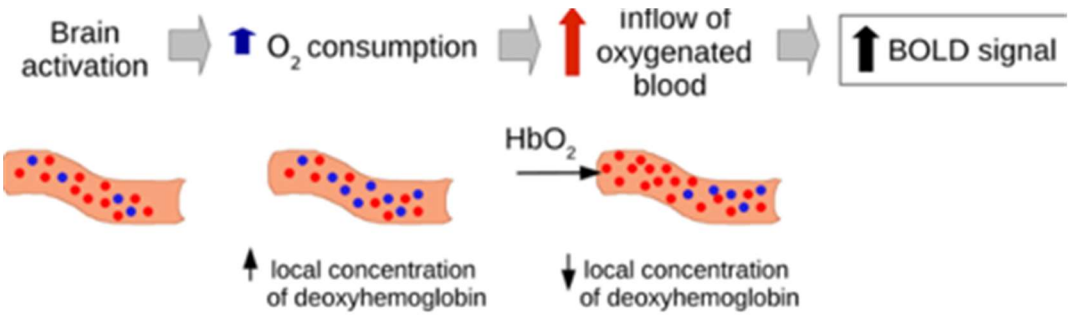


Figure 13. The fMRI BOLD signal. The BOLD signal reflects the changes in deoxyhemoglobin (dHb) concentration in the brain. When brain activity in a local region increases, concomitant increase in oxygen (O_2) consumption triggers upregulation of cerebral blood flow via arteriolar vasodilation. The O_2 supply transiently exceeds demand, which increases local O_2 saturation reducing dHb concentration in veins and capillaries for several seconds. As oxygenated and deoxygenated Hb has different magnetic properties, the above phenomenon causes a BOLD signal increase, which can be captured by T2-weighted sequences.

[Arias J, et al. PyHRF: A Python Library for the Analysis of fMRI Data Based on Local Estimation of the HRF. Published online 2017:34-40. doi:10.25080/SHINMA-7F4C6E7-006]

The mathematical model that represents the neurovascular coupling between local neural activity and the corresponding BOLD signal is called Hemodynamic Response Function (HRF). It describes the expected changes in the BOLD signal following neural activity, providing information about the temporal dynamics of this hemodynamic response *(Figure 14)*. In extreme synthesis, by using the HRF as a model, researchers can examine the time series data obtained from fMRI scans and compare the expected BOLD signal changes based on the HRF model with the observed BOLD signal changes. Assuming that areas with similar BOLD time series are functionally connected, it is possible to quantify the degree of correlation between brain regions and infer the strength of functional connections.

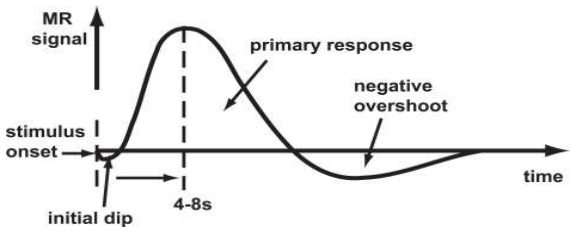


Figure 14. The Hemodynamic Response Function.

Two main approaches can be adopted to acquire fMRI data[161]:

i) *Tasked-based fMRI*: in this approach, neural activity is evoked by asking the subject to perform a task. The task is designed to target a specific motor (e.g., finger tapping) or cognitive process (e.g., mental rotation, verb generation). During the task, fMRI scans are conducted to capture the corresponding BOLD signal changes. By comparing these changes to the baseline activity, researchers can identify localized brain regions that are specifically associated with task performance;

ii) *Resting-state fMRI*: it focuses on the intrinsic activity of the brain while the subject is at rest. The BOLD signal is recorded and analyzed to identify spontaneous low-frequency fluctuations (typically in the range of 0.01 - 0.08 Hz). These fluctuations are believed to reflect intrinsic functional connectivity between brain regions. This approach is data-driven, as it explores patterns of functional connectivity across the whole brain without a priori assumptions, allowing researchers to investigate the organization of large-scale brain networks.

More detailed information about task-based and rs-fMRI approaches is reported in Table 3.

Table 3. Comparisons between task-based and rs-fMRI.

Task-based fMRI	Rs-fMRI
Analyses of the spontaneous modulations in the BOLD signal in the presence of a particular activity (e.g. finger-tapping, eye-blinking, naming, memorizing, etc.)	Analyses of the spontaneous BOLD signal in the absence of any explicit task or an input
Task-related increase in neuronal metabolism are less than 5%	60–80% of brain's energy is consumed during resting state
During task-based activity the focus is only on a very small fraction of the brain's overall activity	In terms of overall brain function, the resting state brain activity is far more significant than task-related activity
The signal during a task-related activity is very small compared to the noise, i.e. 80% of the BOLD modulation is discarded as noise	The signals which are discarded as noise in task fMRI is taken as signals in rs-fMRI as they are the low frequency spontaneous fluctuations in the BOLD signal
Due to discarding of signal as noise, task fMRI has a low SNR	Have improved SNR since it takes the overall spontaneous low frequency fluctuations
For the interpretation of results, a large number of trials are required in task fMRI	No need of more trials like task fMRI
If one wants to analyse the motor function and language function, a separate task may be required to analyse each function in task-based fMRI	In rs-fMRI, the acquired may be used to analyse one or more functions
Patient cooperation is essential to do task fMRI	Paediatric patients, patients with low IQ and even patients in the vegetative and coma state are able to do rs-fMRI
Repeated sessions of task-based activity to assess the disease prognosis, treatment effect etc. will result in familiarity with the task which will affect the output adversely	In rs-fMRI even we are taking different sessions, due to the absence of task, we are able to avoid the task-related confusions and uncertainties faced by task fMRI

SNR: signal-to-noise ratio.

To correlate activity between brain regions in rs-fMRI studies, researchers typically follow these steps[162]:

i) *Acquisition of BOLD signal* across the whole brain over time: since BOLD is a dynamic signal, a "fast acquisition" is provided by Echo-Planar Imaging (EPI) with temporal resolution (TR) ≤ 2 seconds;

ii) *Preprocessing* is needed to clean the data, remove noise from various sources, correct artifacts, and align images to a common coordinate system. It may include slice time correction, distortion correction, and head motion correction, temporal filtering to focus on specific frequency bands of interest, normalization to align data across subjects, spatial smoothing to enhance the signal-to-noise ratio, etc;

iii) *Correlation analysis*: once data are cleaned, several analyses can be conducted:

   i) *Seed-based analysis*: this method requires a priori selection of a region of interest (ROI) or "seed", then the BOLD time series of the seed is correlated with the time series of all other voxels in the brain. The degree of correlation is usually quantified using the r Pearson correlation coefficient for continuous variables *(Figure 15)*[163];

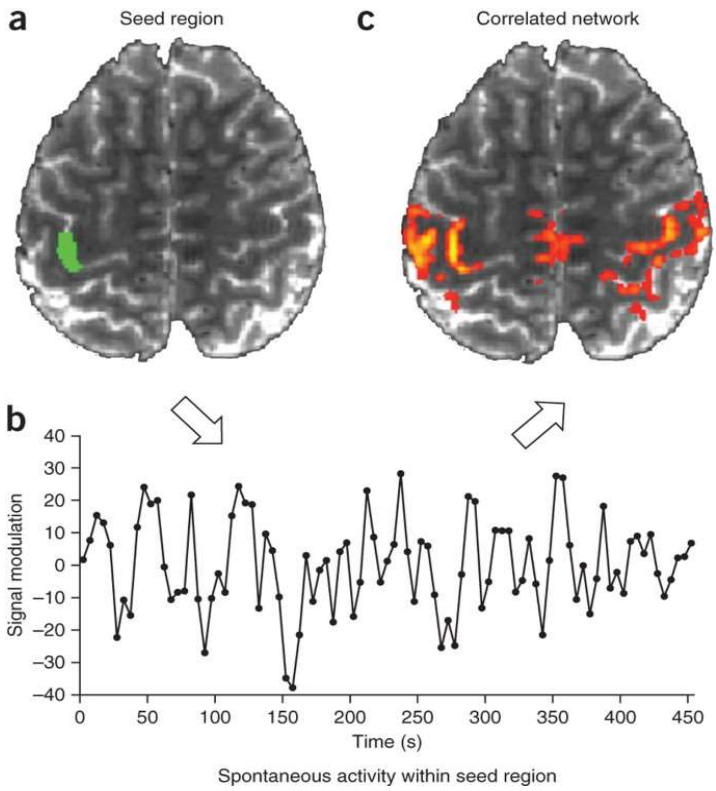


Figure 15. Basic principles of seed-based rs-fMRI. The general strategy of seed-based rs-fMRI is to determine the network of brain regions that show correlated activity fluctuations over time with the seed. **(a)** An example seed region in the motor cortex (green). **(b)** The time course of intrinsic activity fluctuations for the seed region for 7 min. **(c)** Many cortical regions in the motor system are correlated with the seed.

[Buckner RL, Krienen FM, Yeo BTT. Opportunities and limitations of intrinsic functional connectivity MRI. *Nat Neurosci*. 2013;16(7):832-837. doi:10.1038/nn.3423]

An important notion is that the brain can be subdivided into smaller regions or parcels using parcellation schemes, such as anatomical (e.g., Automated Anatomical Labelling AAL3[164]) and functional atlases (e.g., Schaefer atlas[165], which offers a range of options from 100 to 1000 parcels defined by their functional connectivity).

The correlation coefficients obtained from the correlation analysis are used to populate the correlation matrix, which can be displayed as a heatmap *(Figure 16)*.

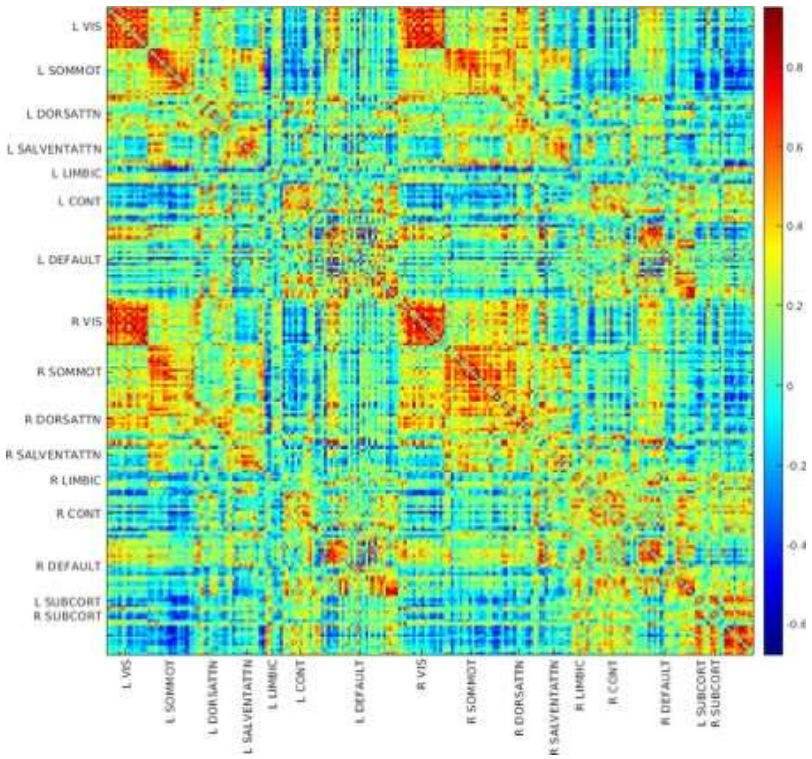


Figure 16. Example of a correlation matrix. The correlation matrix is arranged as a square grid, where rows and columns of the matrix correspond to the parcels being considered and each cell represents the correlation coefficient between two parcels. The diagonal cells usually have a constant value of 1, representing the perfect correlation of a parcel with itself. The matrix is a heatmap that uses colors to represent the magnitude of correlation coefficients. Typically, a color gradient is employed, where warmer colors (e.g., yellow, orange, red) indicate positive correlations whereas cooler colors (e.g., blue, green) represent negative correlations.

ii) *Independent component analysis* (ICA): this method involves an exploratory multivariate data-driven approach. It decomposes the MRI dataset into statistically independent components, each representing a spatial pattern of brain activity and its corresponding time course. Correlation analysis can be performed on the time courses of these independent components to explore functional connectivity networks without a priori seed selection. Thanks to this approach the cortical functional networks have been identified for the first time[166] *(Figure 17)*.

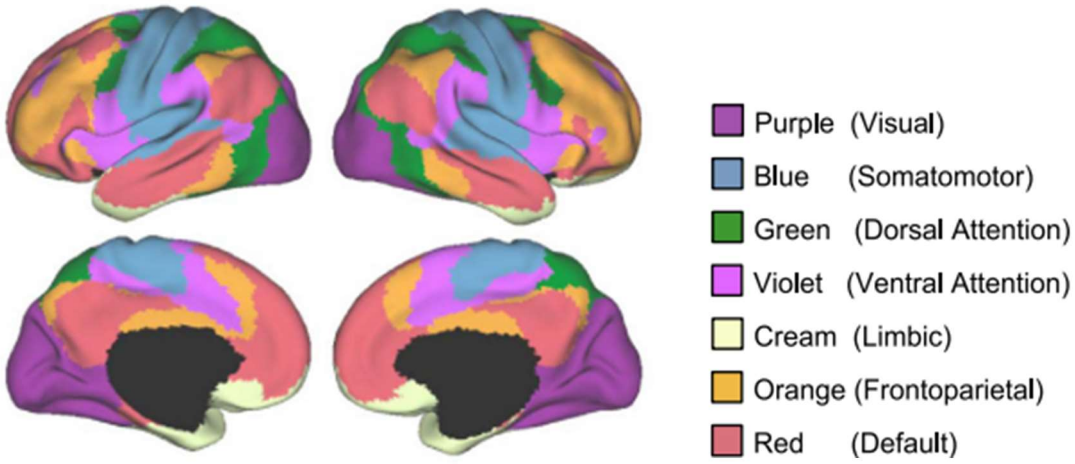


Figure 17. Seven cortical functional networks. Yeo et al.[167] explored the organization of networks in the human cerebrum using rs fMRI. On the right, a table of colors is assigned to the proposed networks and their common names from neuroimaging literature.

[Thomas Yeo BT, Krienen FM, Sepulcre J, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J Neurophysiol*. 2011;106(3):1125-1165. doi:10.1152/jn.00338.2011]

Noteworthy is that these cerebral networks map onto the cerebellum with topographic specificity. The motor networks map onto sensorimotor parts of the cerebellum in the anterior lobe and lobule VIII. Conversely, the dorsal attention, frontoparietal/executive control, ventral attention/salience, and default mode networks map onto focal areas within the cerebellar posterior lobe[168–171]. Similarly, dorsal and ventral subregions of the dentate nucleus also exhibit functional connectivity differences, with the dorsal part showing functional connectivity with the anterior cerebellum and primary motor cortex, and the ventral dentate showing functional connectivity with cerebellar crus I (part of Lobule VII) and the prefrontal cortex[172].

In a large-scale study involving 500 healthy young adults, with a replication sample of another 500 healthy young adults, Buckner et al.[173] *(Figure 18)* examined the functional connectivity between the cerebellar cortex and either 7 or 17 cerebral cortical networks based on a winner-takes-all approach: each cerebellar voxel was mapped based on the particular cortical network that exhibited the highest correlation with its rs-BOLD signal. This revealed extensive functional connectivity between the cerebellum and the cerebral cortex – all brain regions were represented in the cerebellum, except for the primary visual cortices.

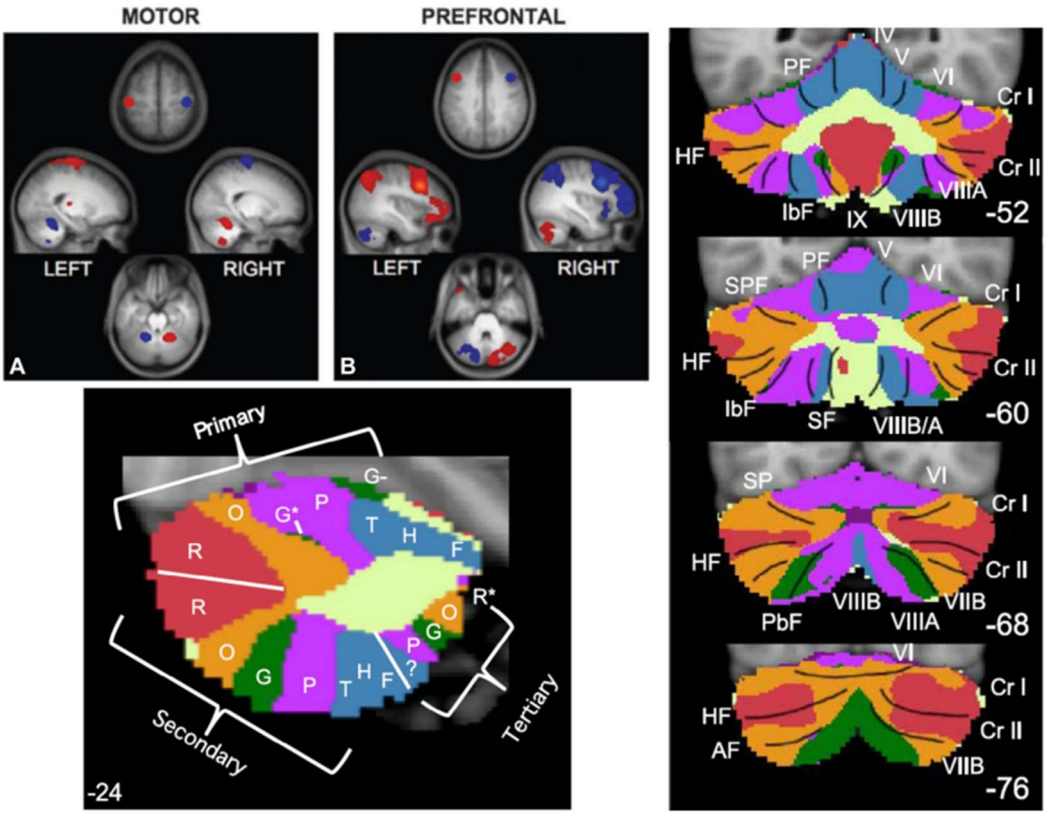


Figure 18. Functional connectivity mapping reveals cerebellar functional topography.
Top left, seeds in the primary motor cortex (A) are contralaterally functionally correlated with the anterior cerebellum and lobule VIII, whereas prefrontal seeds (B) show functional correlations with the posterior cerebellum.
Right and bottom left, functional connectivity patterns in the cerebellum based on cerebral cortical functional networks show sensorimotor network representation (blue) in the anterior lobe and lobule VIII (F foot, H hand; T tongue), whereas ventral attention (purple, P), dorsal attention (green, G), frontoparietal (orange, O), and default-mode (red, R) networks map to posterior cerebellar hemispheres. The data suggest that there are at least two, and potentially three, complete maps of the cerebral cortical networks in the cerebellum (bottom left).

[Adapted from: Buckner RL, Krienen FM, Castellanos A, et al. (2011) The organization of the human cerebellum estimated by intrinsic functional connectivity. J Neurophysiol 106: 2322–2345.]

The realization that the most of human cerebellum is linked with cerebral association areas concerned with intellect, social cognition, and emotion control represents a significant revolution in our understanding of the cerebellum and the organization of the human brain[148].

Task-based fMRI studies conducted on individual subjects and at group level provide independent confirmation of the motor/cognitive dichotomy within the cerebellum[150,174]. Tasks such as limb movement activate the primary sensorimotor cerebellum (i.e., anterior lobe and adjacent parts of lobule VI), as well as the second sensorimotor area in lobule VIII.

In contrast, cognitive paradigms engage distinct regions within the cerebellar posterior lobe. For instance, lobules VI and Crus I are involved in language and verbal working memory; lobule VI in spatial tasks; lobules VI, Crus I, and VIIB are activated by executive functions such as working memory, planning, organizing, and strategy formation; and vermal lobules VI and VII are involved in emotional processing. Language tasks predominantly exhibit right-lateralization, while spatial functions show left-lateralization, reflecting the crossed connections between the cerebellum and cerebral cortex.

Recent rs-fMRI and task-based MRI analysis from the Human Connectome Project (HCP) dataset (n =787 subjects)[175] confirm the dual motor representation in the anterior lobe and lobule VIII. They also confirm and extend the findings of Buckner et al.[173] by demonstrating a triple representation of cognitive domains in the cerebellar posterior lobe, specifically i) lobules VI-Crus I, ii) Crus II-VIIB, and iii) lobule IX. Further analyses of rs-fMRI in the HCP dataset (n = 1003 subjects)[176] reveal that the cerebellum exhibits the same kind of sensorimotor-to-cognition hierarchical gradient pattern that has long been established in the cerebral cortex[177].

Ultimately, functional connectivity studied with fMRI has the potential to be an imaging biomarker of cognitive performance in neurodegenerative disease and is the subject of a growing research field in MS. Such a marker could offer a fast, non-invasive way to detect imminent cognitive decline, which is often underdiagnosed on routine neurological examinations[178].

1.3.3. Clinical evidence of a cerebellar role in cognition

The understanding of the fundamental organizing principles of cerebellar structure and function described in the previous sections aligns with the advancements in the field of clinical neurology related to the cerebellum.
Cerebellar functional topography becomes evident when examining the effects of cerebellar damage or disease in both pediatric[179] and adult[180,181] populations.
Damage to the anterior regions of the cerebellum is associated with cerebellar motor syndrome, which is characterized by impairment of balance and gait ataxia, limb dysmetria, dysarthria, and oculomotor disorders[182]. This syndrome has an anatomical signature, as determined by studies of patients with focal cerebellar lesions. For instance, studies have shown that limb and gait ataxia are more strongly associated with stroke in the territory of the superior cerebellar artery (SCA) territory rather than the posterior inferior cerebellar artery (PICA)[183].

A voxel-based morphometric study of patients with cerebellar lesions showed significant correlations between scores on the International Cooperative Ataxia Rating Scale (ICARS) and damage to the anterior lobe (lobule II-V, extending to lobule VI)[184]. In addition., a recent study investigating the relationship between lobular volumes and motor and cognitive measures in patients with cerebellar disease showed that anterior-lobe and lobule VI volumes were associated with motor function[185]. In MS patients, anterior (but not posterior) lobe volume was an independent predictor of peg-moving performance[186]. All these findings support the idea that the anterior lobe and lobule VI are involved in sensorimotor circuitry.

Similarly, damage to the deep cerebellar nuclei, particularly the interpositus, the fastigial, and the dorsal dentate nuclei can lead to predictable motor outcomes, as seen in children and adolescents following the removal of cerebellar tumors[187].

In contrast, cognitive deficits are more commonly associated with damage to the posterior cerebellum or the ventral dentate nucleus.

The recognition of the “Cerebellar Cognitive Affective Syndrome” (CCAS) or Schmahmann’s syndrome in both adults[188] and children[189] has established the clinically relevant parameters of the non-motor cerebellar function.

Consistent with the anatomic connectivity and functional activation patterns in the posterior cerebellum, the CCAS is characterized by deficits in i) executive function, ii) visuospatial cognition, iii) language, and iv) emotion–affect following cerebellar damage. It is postulated to reflect "dysmetria of thought" analogous to the dysmetria of motor control from damage to the motor cerebellum[148].

It is important to note that cerebellar motor syndrome can exist in the absence of the CCAS, and vice versa the CCAS can be present without motor symptoms. In a recent lesion symptom-mapping study, patients with cerebellar motor syndrome but no cognitive deficits showed damage to the anterior lobe with spared posterolateral hemispheres; the opposite pattern was seen in patients with CCAS but normal ataxia scores[181] (*Figure 19*). This double dissociation between motor and cognitive sequelae suggests that cognitive outcomes in cerebellar damage are independent of motor impairment.

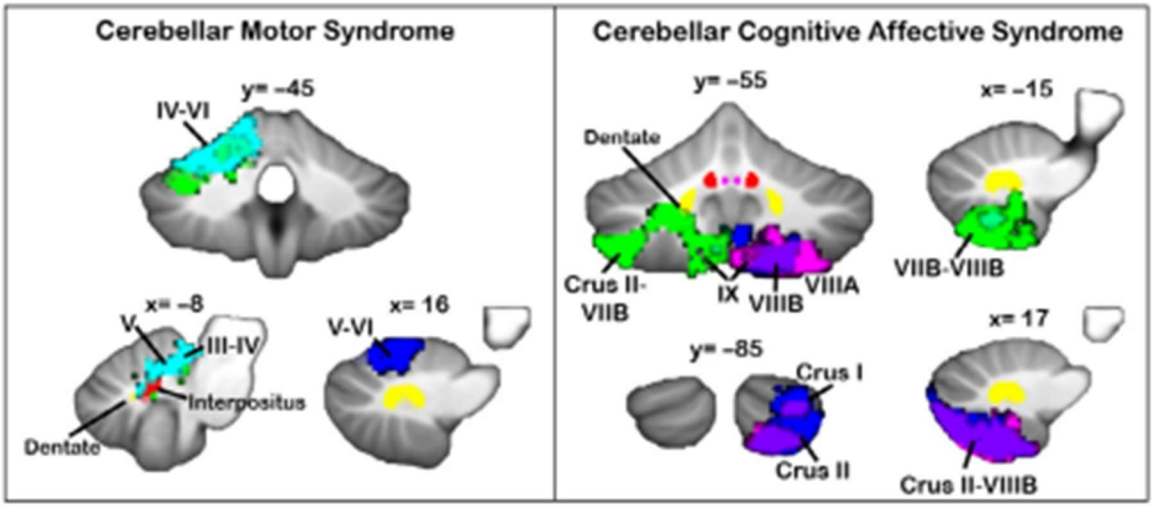
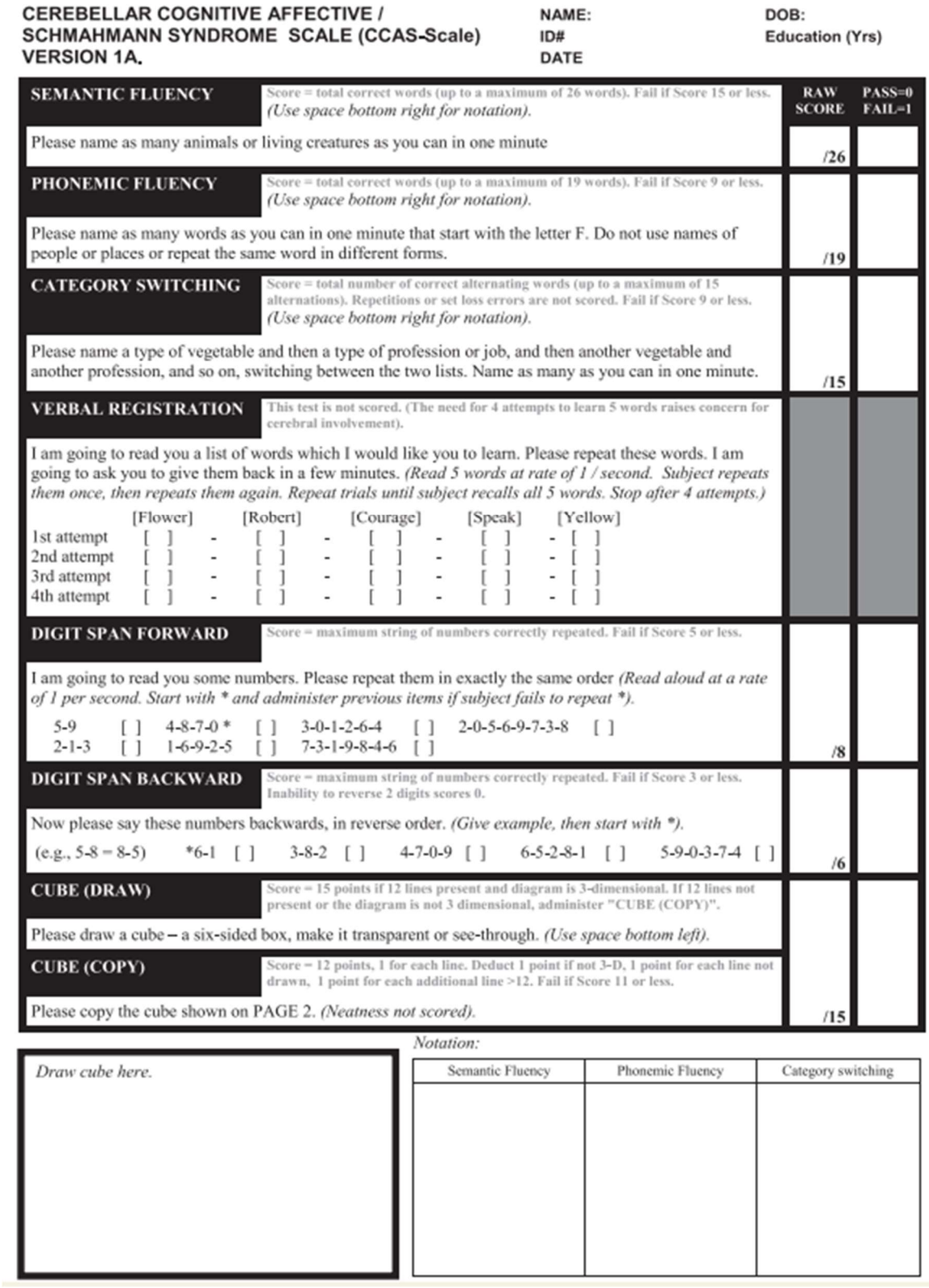


Figure 19. **Lesions associated with cerebellar motor syndrome (left) and CCAS (right).** Different colors represent individual patients' lesions. The patients with cerebellar motor syndrome did not have cognitive deficits, and the patients with CCAS did not have motor deficits. The cerebellar deep nuclei are shown in yellow (dentate), red (interpositus), and violet (fastigial) based on the Spatial Unbiased Infratentorial Template (SUIT) atlas.

[Adapted from Stoodley CJ, Macmore JP, Makris N, et al. (2016) Location of lesion determines motor vs. cognitive consequences in patients with cerebellar stroke. Neuroimage Clin 12: 765–775.]

In 2018 the "CCAS/Schmahmann syndrome scale", a 10-min battery of cross-domain assessments, was validated for CCAS diagnosis in adult patients with cerebellar lesions *(Figure 20)*[190].



CEREBELLAR COGNITIVE AFFECTIVE / SCHMAHMANN SYNDROME SCALE (CCAS-Scale) VERSION 1A.

NAME: ID# DATE

DOB: Education (Yrs)

		RAW SCORE	PASS=0 FAIL=1
SEMANTIC FLUENCY	Score = total correct words (up to a maximum of 26 words). Fail if Score 15 or less. *(Use space bottom right for notation).*		
Please name as many animals or living creatures as you can in one minute		/26	
PHONEMIC FLUENCY	Score = total correct words (up to a maximum of 19 words). Fail if Score 9 or less. *(Use space bottom right for notation).*		
Please name as many words as you can in one minute that start with the letter F. Do not use names of people or places or repeat the same word in different forms.		/19	
CATEGORY SWITCHING	Score = total number of correct alternating words (up to a maximum of 15 alternations). Repetitions or set loss errors are not scored. Fail if Score 9 or less. *(Use space bottom right for notation).*		
Please name a type of vegetable and then a type of profession or job, and then another vegetable and another profession, and so on, switching between the two lists. Name as many as you can in one minute.		/15	
VERBAL REGISTRATION	This test is not scored. (The need for 4 attempts to learn 5 words raises concern for cerebral involvement).		
I am going to read you a list of words which I would like you to learn. Please repeat these words. I am going to ask you to give them back in a few minutes. *(Read 5 words at rate of 1 / second. Subject repeats them once, then repeats them again. Repeat trials until subject recalls all 5 words. Stop after 4 attempts.)*			
[Flower] [Robert] [Courage] [Speak] [Yellow]			
1st attempt [ ] - [ ] - [ ] - [ ] - [ ]			
2nd attempt [ ] - [ ] - [ ] - [ ] - [ ]			
3rd attempt [ ] - [ ] - [ ] - [ ] - [ ]			
4th attempt [ ] - [ ] - [ ] - [ ] - [ ]			
DIGIT SPAN FORWARD	Score = maximum string of numbers correctly repeated. Fail if Score 5 or less.		
I am going to read you some numbers. Please repeat them in exactly the same order *(Read aloud at a rate of 1 per second. Start with * and administer previous items if subject fails to repeat *).*			
5-9 [ ] 4-8-7-0 * [ ] 3-0-1-2-6-4 [ ] 2-0-5-6-9-7-3-8 [ ]			
2-1-3 [ ] 1-6-9-2-5 [ ] 7-3-1-9-8-4-6 [ ]		/8	
DIGIT SPAN BACKWARD	Score = maximum string of numbers correctly repeated. Fail if Score 3 or less. Inability to reverse 2 digits scores 0.		
Now please say these numbers backwards, in reverse order. *(Give example, then start with *).*			
(e.g., 5-8 = 8-5) *6-1 [ ] 3-8-2 [ ] 4-7-0-9 [ ] 6-5-2-8-1 [ ] 5-9-0-3-7-4 [ ]		/6	
CUBE (DRAW)	Score = 15 points if 12 lines present and diagram is 3-dimensional. If 12 lines not present or the diagram is not 3 dimensional, administer "CUBE (COPY)".		
Please draw a cube – a six-sided box, make it transparent or see-through. *(Use space bottom left).*			
CUBE (COPY)	Score = 12 points, 1 for each line. Deduct 1 point if not 3-D, 1 point for each line not drawn, 1 point for each additional line >12. Fail if Score 11 or less.		
Please copy the cube shown on PAGE 2. *(Neatness not scored).*		/15	

Draw cube here.

Notation:

Semantic Fluency	Phonemic Fluency	Category switching

Figure 20. The CCAS scale (Version 1A). The CCAS scale is a screening instrument to detect CCAS in patients with cerebellar injury. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuospatial abilities, abstract thinking, and affect. The total possible raw score is 120 points. The Pass / Fail measure provides a maximum fail score of 10 (i.e., 10 failed tests). *(continued)*

VERBAL RECALL	Spontaneous = 3 points per word, category = 2 points , multiple choice = 1 point. Score = total points. Fail if Score 10 or less. Inability to recall more than 1 word from multiple choice raises concern for cerebral involvement.	RAW SCORE	PASS=0 FAIL=1
What were the words I asked you to learn earlier? *(Subject recalls the words learned previously. Use cues and multiple choice alternatives bottom left if needed).*			
	[Flower] [Robert] [Courage] [Speak] [Yellow]		
Spontaneous recall:	[ ] - [ ] - [ ] - [ ] - [ ]		
Recall with category cue:	[ ] - [ ] - [ ] - [ ] - [ ]		
Recall with multiple choice:	[ ] - [ ] - [ ] - [ ] - [ ]	/15	
SIMILARITIES	Correct answer (conceptual) = 2 points, partial answer (concrete) = 1 point, incorrect answer / no answer = 0 points. Score = total points. Fail if Score 6 or less. Key-bottom right.		
How are the following words alike; what is the same about them? (*Provide example, then test items).*			
(e.g., Ball/Moon = Round)	1.Nose/Ear [__/2] 2. Sheep/Elephant [__/2] 3. Lake/River [__/2] 4. Airplane/Motorcycle [__/2]	/8	
GO NO-GO	2 points for no errors, 1 point for one error, 0 points for two or more errors. Score = total points. Fail if Score 0.		
I am going to tap the table. When I tap once, please raise your finger then put it back down again. When I tap twice, don't do anything. *(Give an example of each condition to make sure subject understands).*			
1 - 1 - 1 - 2 - 2 - 1 - 2 - 2 - 2 - 1 - 2 - 1 - 2 - 1		/2	
AFFECT	Score 6 points if none are present. Subtract 1 for each item present. Fail if Score 4 or less. *(Rater assesses if the following are present, incorporating input from patient and/or caregiver)*		
[ ] Difficulty with focusing attention or mental flexibility			
[ ] Emotionally labile, incongruous emotions, appears hopeless or depressed			
[ ] Shows easy sensory overload or avoidant behaviors			
[ ] Expresses illogical thoughts or paranoia			
[ ] Lacks empathy, is apathetic, or has blunted affect			
[ ] Angry or aggressive, irritable, oppositional, difficulty with social cues and social boundaries		/6	
	TOTAL SCORE	/120	/10
	Calculate total raw score (1st column) and total number of failed tests (2nd column). 1 failed test = Possible CCAS; 2 failed tests = Probable CCAS; 3 or more failed tests = Definite CCAS		

Copy the cube here.

CUES AND MULTIPLE CHOICE ITEMS FOR VERBAL RECALL TEST					
Test word	**Flower**	**Robert**	**Courage**	**Speak**	**Yellow**
Cue	Grows in the garden	Boy's name	Trait or virtue	Way of communicating	Color
Multiple choice items	Tree	Stephen	Bravery	Speak	Red
	Bush	Michael	Courage	Talk	Green
	Flower	Joseph	Honesty	Sing	Blue
	Grass	Robert	Patience	Shout	Yellow

SIMILARITIES	**Correct conceptual answers (examples)**	**Partial correct / concrete answers (examples)**
Nose/Ear	Sense organs	Face, body part
Sheep/Elephant	Mammals, animals	Legs, tails
Lake/River	Bodies of water	Wet, cold, swim
Airplane/Motorcycle	Vehicles, transportation	Use fuel, ride them

(continued) A fail score of 0 is normal. In a patient with cerebellar disease, a fail score of 1 indicated Possible CCAS, 2 indicates Probable CCAS and 3 or more indicated Definite CCAS.

[Hoche F, Guell X, Vangel MG, Sherman JC, Schmahmann JD. The cerebellar cognitive affective/Schmahmann syndrome scale. *Brain*. 2018;141(1):248-270. doi:10.1093/brain/awx317]

1.3.4. Theories about cerebellum functioning

How does the cerebellum modulate cognition? To tackle this question more directly, two main theories have been proposed *(Figure 21)*:

i) The universal cerebellar transform[191]: this theory was proposed by Schmahmann in 1996. It suggests that a singular functional module emerges from an essentially uniform cerebellar circuit and performs computations at different levels of information processing. According to this theory, the cerebellum contributes to diverse functions by applying a general computational principle;

ii) The multiple functionality hypothesis[192]: in contrast to the previous one, this theory suggests that the same uniform circuit can be used to realize various computations relying on variable contributions from distinct cerebellar functional modules. In this view, different modules within the cerebellum are involved in different cognitive processes.

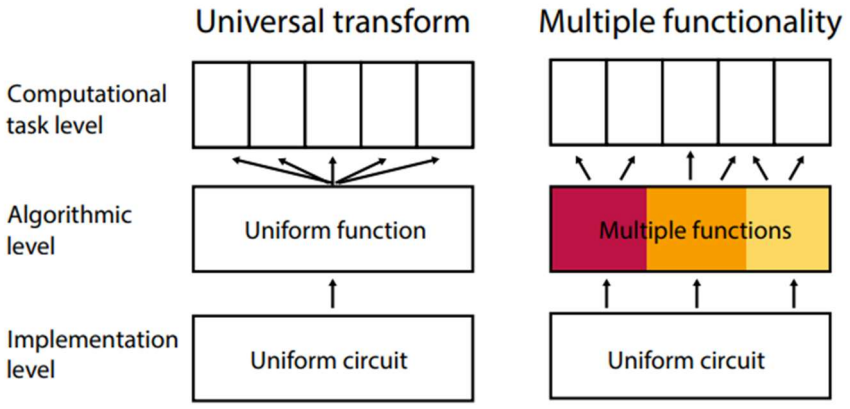


Figure 21. Schematic of the Universal Transform and Multiple Functionality Hypotheses, Considered across Marr's Three Levels of Analysis[193]. At the computational level, each task demands a different computational description. At the implementation level, the cerebellar circuitry is remarkably uniform. The idea of a universal transform holds that, at the algorithmic level, we can formulate a general idea of how cerebellar circuits contribute to diverse functions. In contrast, the multiple functionality models posits that different tasks rely on variable contributions from several cerebellar functional modules, each of which requires a distinct algorithmic description.

[Diedrichsen J, King M, Hernandez-Castillo C, Sereno M, Ivry RB. Universal Transform or Multiple Functionality? Understanding the Contribution of the Human Cerebellum across Task Domains. Neuron. 2019;102(5):918-928.]

From a clinical standpoint, these theories have potential implications in predicting the impact of cerebellar degeneration on a function level. Several scenarios may be hypothesized: for instance, following the universal cerebellar transform, it is plausible to expect that the degenerative processes may induce nonspecific effects at a computational level, resulting in a general disruption of the domain that interacts with the damaged system. Conversely, an alternative scenario, according to the multiple functionality hypothesis, could exhibit a less generalized impairment as only a specific functional module may be affected by the degenerative processes. Also, other alternative situations could be outlined, considering the two theories as not mutually exclusive frameworks.

However, it is challenging to translate this knowledge into concrete solutions. At present, the contribution of these theories is much more theoretical rather than practical. Further research is needed to better understand how the cerebellum modulates cognition and provide a rationale for developing new interventions in the clinical context[154].

1.3.5. Cerebellar damage in MS

The cerebellum is a primary site of MS pathology, involving both cerebellar WM and GM[194]. Several studies have described the association between cerebellar changes and symptoms such as ataxia [195,196], tremor [197,198], depression [199], and CI [195,200] in MS, but almost all have considered the cerebellum as a whole.

Recent MRI studies have provided mounting evidence of the cerebellum's role in cognition also in MS patients. Reduced total cerebellar volume has been associated with worse performance on cognitive tests [201]. Another study has found a correlation between increased posterior fossa lesion volume and slowed information processing efficiency[202].

A recent study using ^{11}C-PBR28 MR-PET and 7T MRI showed that MS patients had increased neuroinflammation in the cerebellum, as indicated by elevated translocator protein (TSPO) expression, a marker of microglia activation. This marker also correlated with decreased CPS and neurological disability[203].

Using 7T MRI, cerebellar lesions were detected in the majority of patients, with RRMS patients showing a prevalent involvement of the WM while SPMS patients had a higher prevalence of cortical and leukocortical plaques.

However, the compartmentalization of the cerebellum in motor and cognitive lobes has not been applied to most previous MS studies. Also, no study has characterized the distribution of MS neuroinflammation and lesion load between these lobes. This would be interesting to better understand the involvement of different cerebellar regions and their clinical impact on MS patients.

Moreover, conventional MRI may not be sufficient to disclose all MS-related pathological changes, especially in early disease stages. There are microstructural abnormalities in neuroaxonal integrity and cortical connectivity[204], undetectable by routinary MRI sequences, which may be revealed by means of diffusion MRI (dMRI) for WM abnormalities and resting state functional MRI (rs-fMRI) for GM alterations. Examining the structural and functional connectivity between the cerebellum and cortical areas associated with cognitive functions could shed light on the pathological mechanisms underlying cerebellar damage-related CI in MS. To this regard, a preliminary study based on probabilistic tractography MRI found that a reduction of the structural connectivity between the default mode network (DMN) and the cerebellum correlated with worse CPS performance[205].
Additionally, in early MS, rs-fMRI might detect microstructural WM and GM changes in the cerebellum and its disconnection with cortical brain areas, providing increased sensitivity in evaluating the clinical impact of cerebellar pathology.

Unfortunately, the largest part of previous MS studies did not adopt extensive neuropsychological tests, so they might not be able to detect the complex CCAS described when cerebellar damage occurs, especially in the early phases of MS.
CCAS scale may be a valuable cognitive tool to intercept CI in MS patients with cerebellar involvement. To date, the CCAS scale has never been used to test MS patients stratified for the extension of cerebellar involvement [206].

2. Aim of the study

The aims of this study are:

i) to investigate, by means of 3 Tesla MRI, the association of MRI structural and functional abnormalities of the cognitive cerebellum with the CI in a cohort of very early RRMS patients;

ii) to test the sensitivity and specificity of the CCAS scale, compared to other neuropsychological tests, in detecting cognitive dysfunction in this cohort of MS patients.

3. Research plan and methods

3.1. Study Population

We designed an explorative, cross-sectional study.

Between June 2022 and May 2023, 37 patients diagnosed with RRMS within 4 years from the clinical onset were consecutively enrolled at the MS Center of the Veneto Region at the University Hospital of Padua, Italy. 4 age- and sex-matched healthy subjects (HC) were recruited as control population.

Inclusion criteria were: i) MS diagnosis according to 2017 revised McDonald criteria, ii) Age between 18-50 years, iii) ≥ 8 years of schooling, and iv) Absence of ongoing or history of disease-modifying therapy.

Exclusion criteria were: i) Steroid treatment within 2 months prior to the study (if enrolled subjects needed to receive steroids, all imaging procedures were performed before steroid treatment), ii) Any acute and/or chronic medical conditions potentially affecting the cerebellar structure/functions, iii) History of cognitive/psychiatric disorder, and iv) General MRI exclusion criteria.

A cutoff of 89 points was computed by means of the formula (mean – 1.5 SD) based on a 50 HC sample selected from an ongoing study to validate the CCAS scale in Italian. The enrolled patients were subsequently divided into two different groups according to their CCAS raw score (max total raw score = 120 points). Out of the 37 patients, 26/37 (70%) scored ≥89 points on the CCAS scale and were classified as "Normal-CCAS", while the remaining 11/37 (30%) obtained <89 points and were classified as "Impaired-CCAS".

3.2. Clinical assessment

An extended neuropsychological assessment was administrated by a certified psychologist of the MS center, consisting of:

i) Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS)[207,208];

ii) Delis-Kaplan Executive Function System Sorting Test (D-KEFS ST) [209];

iii) CCAS scale[206];

iv) Beck Depression Inventory-II (BDI-II)[210];

v) Fatigue Severity Scale (FFS) [211].

Physical disability was assessed using the Expanded Disability Status Scale (EDSS).

3.3. Imaging data acquisition

RRMS and HC underwent MRI on 3-Tesla scanner (Ingenia, Philips Medical Systems, Best, The Netherlands) with a 33 mT/m power gradient and a 32-channel head coil.

The MRI protocol included 3D T1 and T2-weighted images (1 mm isotropic voxels) for structural analysis; fluid-attenuated inversion recovery (FLAIR) and double inversion recovery (DIR) images for brain WM and GM lesions segmentation; spin-echo echo-planar imaging (EPI)-sequence for rs-fMRI.

3.4. MRI data analysis

Brain and cerebellar lesions were manually segmented by consensus from two expert raters using the program ITK-SNAP (http://www.itksnap.org/pmwiki/pmwiki.php) on FLAIR and DIR images. Brain WM and cortical GM (cGM) were segmented on 3T T1-weighted images with SPM12 applying a 90% threshold.

Masks of the whole cerebellum, cerebellar WM and GM were segmented on 3T T1-weighted images using SPM12. A mask of the cognitive cerebellum was derived by a coregistered-AAL3 atlas, extracting cerebellar lobules VI to X, excluding lobule VIII (which is part of the motor cerebellum).

On rs-fMRI images, seed-based connectivity analysis (SCA) was performed, using the mask of cognitive cerebellum as seed, and Schaefer 100 parcels atlas[165] for defining the brain cortical networks as Regions of Interest (ROIs).

3.5. Statistical analysis

Statistical analyses were performed using SPSS 22.0 (StataCorp LP, College Station, TX, USA). Normality in measurements was tested graphically and using Kolmogorov-Smirnov test. Nonparametric tests were used for non-normal or skewed data and parametric tests for normally distributed data. Respectively, median (interquartile range)

and mean (± standard deviation) are shown. Differences between groups were analyzed using the chi-squared test for categorical variables, either 2-tailed t-test or ANOVA (the latter if comparisons between 3 groups) for parametric continuous variables and the Mann–Whitney test for nonparametric continuous variables (such as structural-functional MRI parameters).

To compare neuropsychological parameters between groups, ANCOVA was performed, setting the test raw score as the dependent variable, and the years of education as the covariate.

To assess the association between the neuropsychological tests (i.e., CCAS scale) and structural-functional MRI parameters, partial correlation corrected for years of education was used.

A p-value of 0.05 was accepted as statistically significant.

4. Results

4.1. Study Population

The demographics and clinical characteristics of our study population are shown in detail in Table 4. The "Normal-CCAS MS" and "Impaired-CCAS MS" groups were comparable in terms of age (p=0.153), years of education (p=0.240), and EDSS (p=0.056). A statistically significant difference was found regarding gender (p=0.004) and disease duration (p=0.005).

Table 4. Baseline Demographics and Clinical Characteristics

	HC	MS	HC vs MS p-value	Normal-CCAS MS	Impaired-CCAS MS	Normal vs Impaired-CCAS MS p-value
Subjects	4	37	-	26	11	
Age, years, mean (SD)	35.7 (11.0)	39.6 (12.8)	0.369[a]	36.8 (12.1)	45.6 (12.8)	0.153[b]
Female, n (%)	2 (50%)	22 (59%)	0.715[c]	20 (77%)	2 (18%)	**0.004**[c]
Years of education, years, mean (SD)	16.9 (2.9)	14.6 (3.8)	0.495[a]	15.3 (3.4)	13.0 (4.2)	0.240[b]
EDSS, median (IQR)	-	1.5 (1.0)	-	1.5 (1.0)	2.0 (2.0)	0.056[d]
Disease duration, year, mean (SD)	-	0.68 (1.08)		0.42 (0.98)	1.27 (1.10)	**0.005**[d]

Significance testing:
[a]2-tailed t-test on means
[b]One-way ANOVA (p-value are reported after Bonferroni correction)
[c]Chi-squared test
[d]Mann-Whitney test
Bold red indicates a statistically significant difference with a p-value<0.05

HC: healthy controls; MS: multiple sclerosis; CCAS: cerebellar cognitive affective syndrome scale; SD: standard deviation; IQR: inter-quartile range; EDSS: Expanded Disability Status Scale

4.2. Neuropsychological parameter comparisons

Table 5 shows the neuropsychological parameter comparisons between groups.

We tested the cognitive performance of our cohort of very early RRMS patients and HC using the CCAS scale, BICAMS (the results are presented separately into SDMT, CVLT-II, and BVMT-R tests), and D-KEFS ST (the results are presented separately into FSC, FSD and SR tests). All tests were standard tools used in clinical and research settings and together probed a broad range of different sensory, cognitive, and output modalities.

Confounders such as depression and fatigue could be excluded, as none of the patients was found to show symptoms of depression, and the level of cognitive efficiency was unrelated to any metric of the Fatigue Severity Scale.

Table 5. Neuropsychological parameter results and comparisons between groups

	HC	MS	HC vs MS p-value	Normal-CCAS MS	Impaired-CCAS MS	Normal vs Impaired-CCAS MS p-value
CCAS scale, mean (SD)	100.0[a] (7.8)	96.7 (13.2)	0.812	103.7 (7.0)	80.0 (8.1)	**<0.001**
SDMT, mean (SD)	52.5 (12.1)	54.5 (12.9)	0.422	58.1 (8.1)	45.9 (13.5)	0.155
CVLT-II, mean (SD)	59.5 (7.6)	54.8 (9.2)	0.478	58.2 (7.5)	46.7 (8.1)	**0.011**
BVMT-R, mean (SD)	30.8 (3.0)	28.6 (6.4)	0.904	30.8 (3.8)	23.3 (8.2)	0.052
D-KEFS FSC, mean (SD)	13.0 (1.4)	11.0 (2.5)	0.171	11.9 (2.1)	8.9 (2.3)	**0.004**
D-KEFS FSD, mean (SD)	51.8 (5.9)	43.4 (10.3)	0.162	47.0 (8.5)	34.9 (9.4)	**0.004**
D-KEFS SR, mean (SD)	50.0 (6.9)	41.4 (9.5)	0.115	44.8 (7.3)	33.5 (9.7)	**0.004**

Significance testing:
ANCOVA: dependent variable = test raw score; covariate = years of education (for multiple comparisons, p-values are reported after Bonferroni correction)
Bold red indicates a statistically significant difference with a p-value<0.05
HC vs Normal-CCAS MS: no significant difference
[a]HC vs Impaired-CCAS MS: p-value < 0.05
For all tests, the results are expressed as mean (SD) number of correct answers.

HC: healthy controls; MS: multiple sclerosis; SD: standard deviation; CCAS: cerebellar cognitive affective syndrome scale; SDMT: Symbol Digit Modalities Test; CVLT-II: California Verbal Learning Test-II; BVMT-R:Brief Visuospatial Memory Test-Revised; D-KEFS ST:Delis-Kaplan Executive Function System Sorting Test; FSC: Free Sorting Categorization; FSD: Free Sorting Description; SR: Sort Recognition

No statistically significant difference was found between HC and either MS patients (total) or "Normal-CCAS" group. Among patients, the "CCAS-Impaired" group showed significantly lower scores on the CCAS scale (p<0.001), CVLT-II (p=0.011), and D-KEFS FSC (p=0.004), FSD (p=0.004) and SR (p=0.004) compared to "Normal-CCAS" one. Conversely, the results of SDMT and BVMT-R tests did not differ significantly between the two groups.

4.3. Structural MRI parameter comparisons

Table 6 reports the structural MRI parameter comparisons between groups. No statistically significant differences were found between HC and MS patients. Among patients, a significant reduction was found in all cerebellar parameters of the "Impaired-CCAS" group compared to the "Normal-CCAS" group, including cerebellum total volume (p=0.001), WM volume (p=0.013), and cortex volume (p=0.013). The same result was found for other brain structures, i.e., the thalamus (p=0.003) and the corpus callosum (p=0.003), as well as for the whole brain WM volume (p=0.004).

The two MS groups did not show significant differences in whole brain WM lesion volume as well as cognitive cerebellum lesion volume.

Table 6. Conventional MRI parameter comparisons between groups

	HC	**MS**	**HC vs MS p-value**	**Normal-CCAS MS**	**Impaired-CCAS MS**	**Normal vs Impaired-CCAS MS p-value**
WM vol, mean (SD)[x]	3258 (63)	3168 (300)	0.483	3261 (192)	2948 (396)	**0.004**
Cortical Thickness, mean (SD)	4.75 (0.20)	4.62 (0.17)	0.203	4.64 (0.14)	4.58 (0.23)	0.270
Cerebellum total vol, mean (SD)[x]	887 (52)	896 (89)	0.751	923 (7)	832 (10)	**0.001**
Cerebellum WM vol, mean (SD)[x]	161 (11)	168 (21)	0.431	176 (17)	150 (20)	**0.013**
Cerebellum cortex vol, mean (SD)[x]	726 (44)	728 (73)	0.916	747 (61)	682 (81)	**0.013**
Thalamic vol, mean (SD)[x]	49 (5)	45 (7)	0.237	47 (5)	40 (7)	**0.003**
Hippocampus vol, mean (SD)[x]	26 (2)	24 (2)	0.237	25 (2)	23 (5)	0.051
Amygdala vol, mean (SD)[x]	10 (1)	9 (1)	0.337	10 (1)	9 (1)	0.316
Corpus callosum vol (mm^3), mean (SD)[x]	24[a] (3)	21 (4)	0.122	23 (3)	18 (6)	**0.003**
Whole brain WM lesion volume (mm^3), mean (SD)	-	7505 (6676)	-	5685 (4563)	11809 (8912)	0.075
Cognitive cerebellum lesion volume (mm^3), mean (SD)	-	33.3 (68.5)	-	36.0 (77.7)	26.9 (41.3)	0.346
Cerebellar/Extracerebellar lesion volume ratio	-	0.049 (0.158)	-	0.061 (0.188)	0.022 (0.021)	0.087

Significance testing:
Mann-Whitney test
Bold red indicates a statistically significant difference with a p-value<0.05
HC vs Normal-CCAS MS: no significant difference
[a]HC vs Impaired-CCAS MS p-value < 0.05
All structural volumes are normalized for the total intracranial (TIC) volume
[x]expressed as $x10^4$
Cognitive cerebellum includes cerebellar lobules VI, VII, IX, X

HC: healthy controls; MS: multiple sclerosis; CCAS: cerebellar cognitive affective syndrome scale; SD: standard deviation

4.4. Functional MRI parameter comparisons

Table 7 shows the comparisons of functional MRI parameters between the "Normal-CCAS" and "Impaired-CCAS" groups. We opted for a seed-based approach, choosing the cognitive cerebellum mask (lobules VI to X, excluding VIII) as seed and the Schaefer 7-networks (100 parcels) divided into left and right as Regions of Interest (ROIs).

Table 7. Functional MRI parameter comparisons between groups

Seed	Schaefer 7-networks (100 parcels)	Normal-CCAS MS	Impaired-CCAS MS	Normal vs Impaired-CCAS MS p-value
SCA Cognitive cerebellum	R Visual	0.06 (0.08)	0.11 (0.11)	0.101
	R Somatomotor	0.05 (0.07)	0.07 (0.08)	0.332
	R Dorsal Attention	0.09 (0.06)	0.11 (0.07)	0.460
	R Salience	0.07 (0.04)	0.07 (0.06)	0.501
	R Limbic	0.02 (0.02)	0.05 (0.04)	**0.004**
	R Control	0.13 (0.05)	0.14 (0.05)	0.806
	R DMN	0.09 (0.04)	0.11 (0.04)	0.108
	L Visual	0.05 (0.09)	0.11 (0.11)	0.060
	L Somatomotor	0.03 (0.07)	0.08 (0.09)	0.115
	L Dorsal Attention	0.05 (0.08)	0.08 (0.11)	0.181
	L Salience	0.06 (0.04)	0.08 (0.06)	0.192
	L Limbic	0.03 (0.03)	0.05 (0.03)	0.108
	L Control	0.12 (0.06)	0.14 (0.06)	0.332
	L DMN	0.09 (0.04)	0.11 (0.04)	0.094

Correlations with Schaefer 7 networks are expressed as mean (SD) of correlations of parcels included in the single network. Cognitive cerebellum includes cerebellar lobules VI, VII, IX, and X.
Significance testing:
Mann-Whitney test (p-values are reported after FDR correction for multiple comparisons)
Bold red indicates a statistically significant difference with a p-value<0.05

HC: healthy controls; MS: multiple sclerosis; CCAS: cerebellar cognitive affective syndrome scale; SCA: Seed-based Correlation Analysis; R: right; L: left

The only statistically significant difference between the two groups was increased functional connectivity between our seed and the right limbic network (p=0.004), which is involved in several cognitive functions, such as regulating emotional responses, memory formation and retrieval, reward processing, and decision-making. However, although not statistically significant, an increased connectivity pattern between the cognitive cerebellum and the majority of the functional cortical networks was found in the "Impaired-CCAS" group compared to the "Normal-CCAS" one.

This positive tendency is also graphically visible in *Figure 22* and *Figure 23* in which the results are presented, respectively, as boxplots and brain heatmaps.

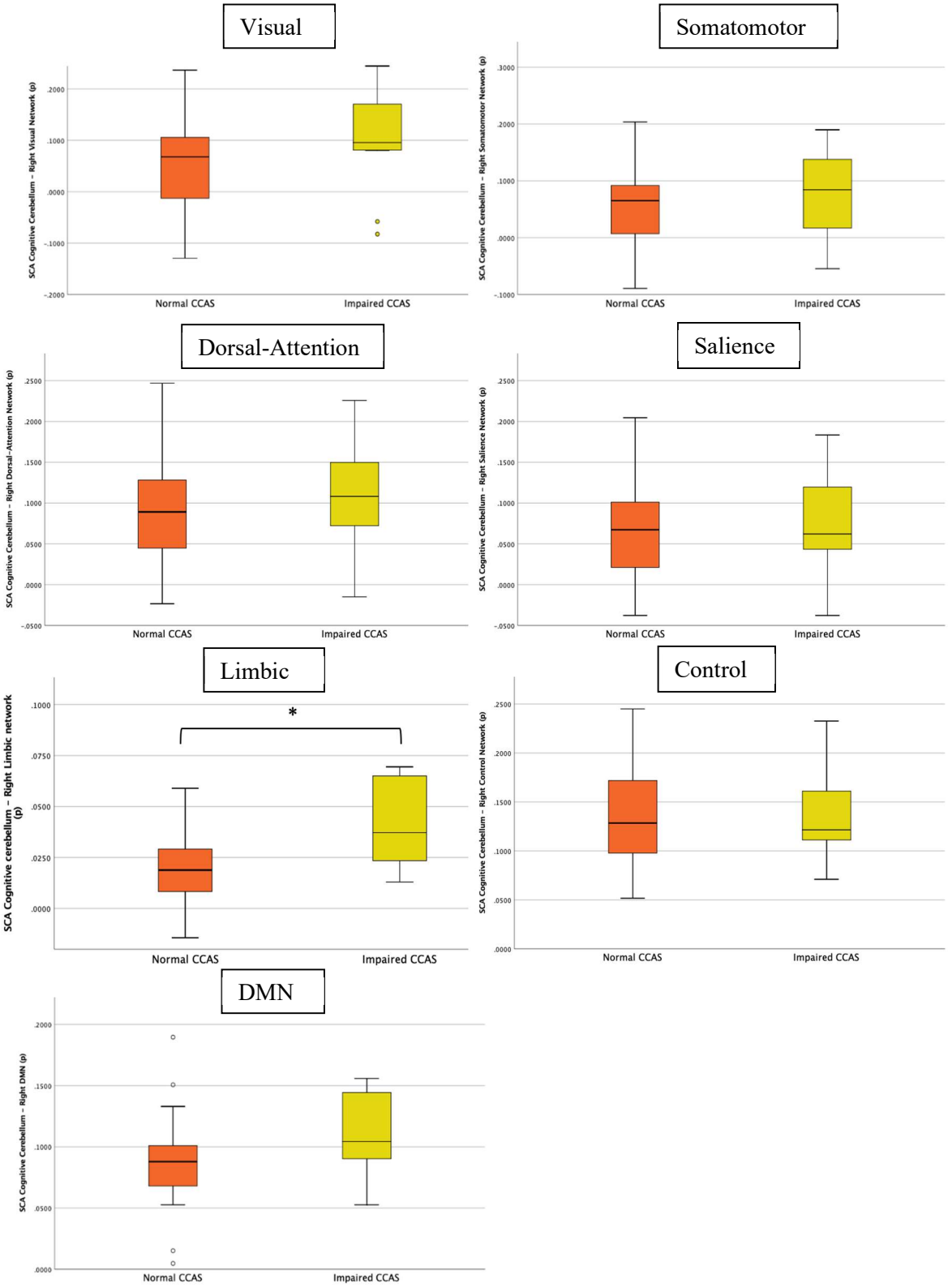


Figure 22. Boxplots representing the comparisons of functional MRI parameters between "Impaired-CCAS" and "Normal-CCAS" groups. The "Impaired-CCAS" group showed stronger connections between cognitive cerebellum and functional cortical networks compared to the "Normal-CCAS" one for most of the correlations, but only the one involving the right limbic network reached statistical significance.

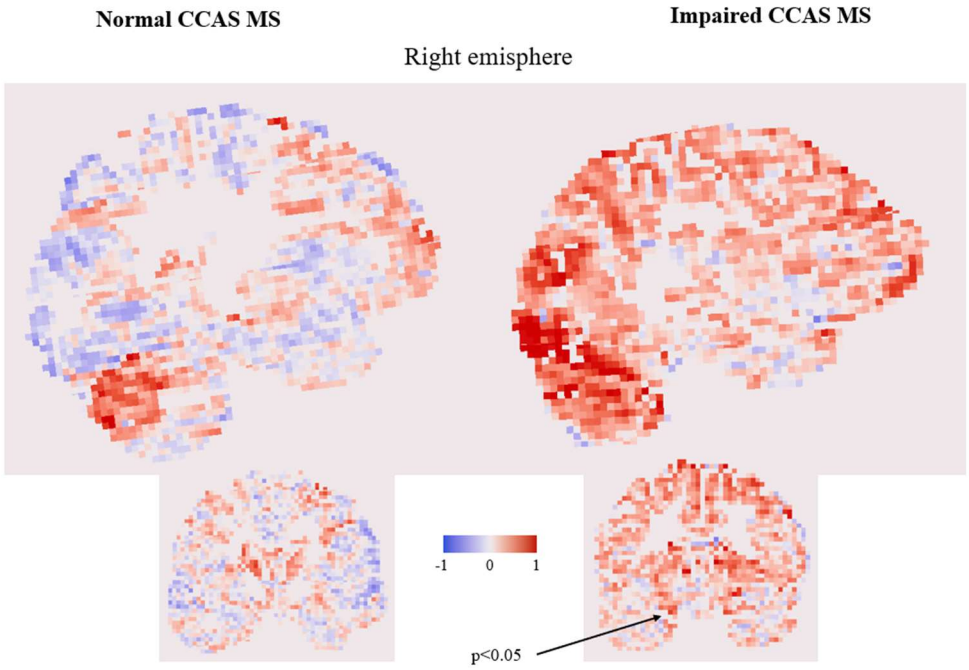


Figure 23. Heatmap representations of the right cerebral hemisphere in the "Normal-CCAS" group (left) and "Impaired-CCAS" group (right). Colours are used to highlight the strength of functional connections between the cognitive cerebellum and different brain regions (red = positive correlation, blue = negative correlation). The "Impaired-CCAS" group showed diffuse cortical hyperactivation compared to the "Normal-CCAS" one.

4.5. Correlations

Tables 8-9 show the results of correlations (corrected for years of education) between cognitive test scores and structural MRI parameters in all MS patients.

For the CCAS scale, statistically significant associations were observed for all the assessed structural MRI parameters, i.e., whole brain WM volume (p=0.006), cerebellum WM volume (p=0.014), thalamic volume (p=0.004), corpus callosum volume (p=0.010), and whole brain WM lesion volume (p=0.027).

For the SDMT test, statistically significant associations were observed for whole brain WM volume (p=0.007), thalamic volume (p=0.010), corpus callosum volume (p=0.011), and whole brain WM lesion volume (p=0.001).

For the CVLT-II test, none of the correlations reached statistical significance.

For the BVMT-R test, statistically significant associations were observed for whole brain WM volume (p=0.043), corpus callosum volume (p=0.018), and whole brain WM lesion volume (p=0.035).

For the D-KEFS SR test, statistically significant associations were observed for all the assessed structural parameters, i.e., whole brain WM volume (p<0.001), cerebellum WM volume (p=0.005), thalamic volume (p<0.001), corpus callosum volume (p=0.025) and whole brain WM lesion volume (p=0.016).

Table 10 shows the results of correlations (corrected for years of education) between CCAS scale score and functional MRI parameters in all MS patients.

Statistically significant differences were found in the correlations between the Cognitive Cerebellum and the Right Limbic Network (p=0.047), the Left Salience Network (p=0.033), and the Left Control Network (p=0.049).

Table 8. CCAS scale score – Structural MRI parameters (all patients)

CCAS scale raw score vs	**r**	**p**
Whole Brain WM vol	0.447	**0.006**
Whole Brain Cortical Thickness	-0.059	0.732
Cerebellum total vol	0.252	0.138
Cerebellum WM vol	0.408	**0.014**
Cerebellum cortex vol	0.187	0.274
Thalamic vol	0.471	**0.004**
Hippocampus vol	0.211	0.217
Amygdala vol	0.214	0.211
Corpus callosum vol	0.422	**0.010**
Whole brain WM lesion volume	-0.370	**0.027**
Cognitive cerebellum lesion volume	0.216	0.205
Cerebellar/Extracerebellar lesion volume ratio	0.183	0.284

Cerebellum WM vol – Thalamic vol correlation: r=0.524, p=0.001
Cerebellum WM vol – Whole Brain WM vol correlation: r=0.469, p=0.003

Table 9. Neuropsychological tests – Structural MRI parameters (all patients)

	Whole Brain WM vol	Cerebellum WM vol	Thalamic vol	Corpus callosum vol	Whole brain WM lesion vol
	p (r)	p (r)	p (r)	p (r)	p (r)
CCAS	**0.006** (0.447)	**0.014** (0.408)	**0.004** (0.471)	**0.010** (0.422)	**0.027** (-0.370)
SDMT	**0.007** (0.442)	0.114 (0.268)	**0.010** (0.426)	**0.011** (0.418)	**0.001** (-0.510)
CVLT-II	0.294 (0.180)	0.146 (0.247)	0.093 (0.285)	0.092 (0.285)	0.366 (-0.155)
BVMT-R	**0.043** (0.339)	0.094 (0.283)	0.051 (0.327)	**0.018** (0.392)	**0.035** (-0.352)
D-KEFS-SR	**<0.001** (0.590)	**0.005** (0.454)	**<0.001** (0.551)	**0.025** (0.372)	**0.016** (-0.397)

Table 10. CCAS scale score – Functional MRI parameters (all patients)

CCAS scale raw score vs	**r**	**p**
Cognitive Cerebellum - R Visual	-0.304	0.071
Cognitive Cerebellum - R Somatomotor	-0.231	0.176
Cognitive Cerebellum - R Dorsal Attention	-0.260	0.126
Cognitive Cerebellum - R Salience	-0.228	0.162
Cognitive Cerebellum - R Limbic	-0.333	**0.047**
Cognitive Cerebellum - R Control	-0.207	0.227
Cognitive Cerebellum - R DMN	-0.299	0.076
Cognitive Cerebellum - L Visual	-0.307	0.069
Cognitive Cerebellum - L Somatomotor	-0.301	0.074
Cognitive Cerebellum - L Dorsal Attention	-0.314	0.062
Cognitive Cerebellum - L Salience	-0.356	**0.033**
Cognitive Cerebellum - L Limbic	-0.157	0.359
Cognitive Cerebellum - L Control	-0.331	**0.049**
Cognitive Cerebellum - L DMN	-0.294	0.081

5. Discussion

Cognitive impairment (CI) affects a large proportion of patients with MS and has a profound impact on their daily-life activities. Understanding the pathophysiology of CI in MS and the mechanisms responsible for its onset and progression over time may contribute to the development of better outcome measures and targets for innovative treatment strategies[27].

Over the past few decades, advancements in investigative imaging techniques and computational modeling systems have revolutionized our knowledge about the cerebellum. These technical improvements have allowed for repeated demonstrations that the cerebellum plays crucial roles in both motor and non-motor functions[143].

Moreover, the description of the "cerebellar cognitive affective syndrome" (CCAS) in individuals with cerebellar damage has provided further evidence of the cerebellum's role in cognition[188]. Recent studies tested the ability of the CCAS scale to diagnose CCAS in patients with acute cerebellar stroke[212] and hereditary ataxia[213], but to date, no study has used the CCAS scale to test patients with MS stratified for the extension of cerebellar involvement.

Based on these considerations, we wondered how cerebellar pathology can contribute to the development of neuropsychological dysfunction in individuals with MS. To address this question, we designed a single-center, exploratory, cross-sectional study using a cohort of very early RRMS patients. We used the powerful and informative tools of conventional and resting-state functional MRI to assess the relation between CI and alterations in cerebellar MRI parameters. To perform the analysis, we divided our cohort of RRMS patients into two groups (i.e., "Normal-CCAS" and "Impaired-CCAS") according to their raw score on the CCAS scale, which we considered an indirect marker of cerebellar damage-related cognitive dysfunction. Secondly, we try to explore the specificity of the CCAS scale for cerebellar MRI damage. To do this, we first analyzed the association between CCAS score and structural-functional MRI measures of MS pathology. Interestingly, CCAS scores did not show only a correlation with cerebellar MRI damage metrics, but also with other cognitive brain structures, such as thalamus, corpus callosum, and brain WM. Additionally, other neuropsychological test scores (i.e., BICAMS and D-KEFS ST) disclosed an association with cerebellar WM pathology.

Taken together, these findings suggest a scarce specificity for the cerebellar damage of the CCAS scale on the MS cognitive dysfunction, probably caused by the disruption of different brain structures and networks which yields a complex and multi-domain CI.

Our two groups of MS patients were comparable for age, years of education, and baseline EDSS, but not for gender since male patients were more represented in the cognitively impaired group compared to the normal one. However, we do not think that this can affect our analysis since no association between gender and cognitive performance has been demonstrated in literature. Interestingly, in our cohort, cognitively impaired patients seem to have a significant diagnostic delay (time between the onset of symptoms and the diagnosis) as they were found to have longer disease duration (mean=1.27 years) compared to the other group (mean=0.42 years). A possible role of the CI (often unrecognized) in the delayed timing of the first neurological examination can be assumed.

Comparing the performance to the different neuropsychological tests, no significant differences were found between healthy controls versus either the total MS sample or the cognitively normal MS group. Noteworthy is that, in early MS patients, the CCAS scale seems to detect deficits in different cognitive domains (i.e., executive function, visuospatial cognition, language, emotion-affection) compared to SDMT (i.e., processing speed), since both patients with normal and impaired-CCAS scale had similar SDMT scores. This finding might have an important impact on clinical practice since demonstrates that SDMT alone (as often administered in clinical practice) might miss a significant amount (up to 30% in our cohort) of cognitive impaired MS patients at the time of diagnosis.

The application of conventional MRI techniques has contributed to improving our understanding of the mechanisms underlying cognitive deficits in patients with MS. Available data suggest that focal lesions do play a role, but the overall impact of T2-weighted lesion load on MS-related CI is limited[26,98,99]. In our study, we assessed the contribution of both whole-brain WM lesion volume and cognitive cerebellum lesion volume on cognitive performance. We found that T2-cerebellar lesion load is similar

between patients with low and high CCAS scores. Thus, we can infer that cerebellar T2-lesion load is not associated with cerebellar-related CI.

This result aligns with existing literature, suggesting that other aspects of MS pathology, such as more subtle, nonfocal WM damage, may play a crucial role in determining the presence and extent of cognitive dysfunction[27]. Indeed, several studies have provided evidence that irreversible tissue loss, measured in terms of global and regional atrophy, is robustly associated with cognitive deficits in MS[103–110]. Consistent with these findings, we observed that the CCAS scale is able to identify MS patients at disease onset with CI who have cerebellar, whole WM, thalamic, and corpus callosum atrophy. However, further studies are needed to assess the exclusive contribution of cerebellar atrophy in the development of MS-related CI.

Correlations between functional connectivity (FC) metrics and cognition have been frequently reported in MS[214–216]. However, when comparing FC between cognitively impaired and cognitively preserved patients, the results have been inconsistent. Some studies have reported both high and low FC associated with worse cognitive performance in MS patients[111–114]. To interpret these contrasting findings, the "network collapse" model has been proposed which postulates three main stages[217]. In the early stage, network efficiency remains normal, because structural damage can be compensated by increases in local activation. This predicts early increases in FC, reflecting these compensatory processes. The second stage occurs when structural damage accrues to a critical point, at which compensatory processes become less effective. Finally, in the third stage, structural damage exceeds the critical point, resulting in a 'network collapse', and concomitant decreases in FC. This functional "reorganization" is a common interpretation in any type of brain function: a compensatory mechanism that enables the functioning of networks in the presence of structural damage, hence delaying clinical progression. Supporting this model, several studies have demonstrated different patterns of FC changes at different disease stages, such as high FC in CIS[218,219], the earliest stage of MS, and low FC in progressive MS[220].

Consistently with these previous findings, our rs-fMRI analysis of a cohort of early-stage RRMS patients revealed that FC between the cognitive cerebellum and most of the functional brain cortical networks (i.e., Limbic, Dorsal Attention, DMN) was higher in

the cognitively impaired group compared to the normal one (even if only in the right limbic network the difference was significant). This finding may possibly be an expression of compensatory hyperactivation of cognitive connections between the cerebellum and brain cortex, promoted by neuroplasticity in response to the microstructural damage of the cerebellum-brain connections.

As further support, a comparative study[218] between patients with CIS and RRMS has suggested that increased resting-state FC might be an early and finite process (present only in CIS and patients with early RRMS), which can become exhausted with the accumulation of disease-related structural damage.

However, it remains unclear whether these increased FC patterns are truly beneficial or maladaptive for cognitive function. Further investigation with longitudinal studies is needed to better understand the progression of the "network collapse" and determine at which point these adaptive changes may become maladaptive.

In summary, our study showed a poor specificity of the CCAS scale, proposed by Schmahmann[190], in identifying cerebellar damage-related CI in patients with early MS. Correlations between neuropsychological tests scores and structural MRI parameters revealed that performance to CCAS scale is positively correlated with WM whole brain, WM cerebellar volume, thalamic and corpus callosum volume, and inversely correlated with T2-whole brain lesion load. Thalamic volume showed the strongest correlation with CCAS scale score, revealing the CCAS scale is not specific for cerebellar-related CI in MS. SDMT, BVMT-R, D-KEFS SR raw scores were also correlated with whole brain WM, corpus callosum volume, and T2-whole brain lesion load, and notably D-KEFS SR was correlated with the same structural MRI parameters as CCAS scale (including cerebellar WM), confirming the CCAS scale assesses cognitive domains controlled not only by the cerebellum but also by other brain structures.

In addition, lower CCAS scale scores were correlated with higher cerebellar-brain FC, especially for Limbic, Salience, and Control networks, as possible expression of compensatory hyperactivation in CI patients.

Our study presents several limitations. First, we did not analyze diffusion-weighted imaging (DWI) data, so the micro-structural analysis could not be included.

Second, the CCAS scale is still not validated in the Italian language, but we used a back-translated Italian version of this scale whose validation is ongoing in a parallel multi-centric Italian project.

Third, the present study is cross-sectional. Longitudinal investigations may shed light on how the accumulation of cerebellar pathology relates to clinical manifestations in MS.

A final methodological limitation is the limited number of healthy subjects recruited as the control comparison group. This represented a problem for the functional analysis, in which the comparison between MS patients and HC was not included due to high inter-subject variability. A more extensive enrollment in both our samples is needed in order to complete the functional analysis.

6. Conclusion

In conclusion, our findings suggest that CI in the early stages of RRMS is associated with pathological alterations in both structural and functional MRI parameters. Higher FC between cerebellar-brain networks in CCAS-impaired patients might be the expression of a compensatory hyperactivation of altered cognitive cerebellar connections. Finally, although the CCAS scale has proven able to detect CI in MS patients, though the CCAS scale has proven able to detect CI in MS patients, its specificity for cerebellar pathology in early MS needs to be investigated in a larger cohort.

Bibliography

1. Filippi M, Bar-Or A, Piehl F, et al. Multiple sclerosis. *Nat Rev Dis Primers*. 2018;4(1). doi:10.1038/s41572-018-0041-4
2. Mapping multiple sclerosis around the world key epidemiology findings Atlas of MS 3 rd edition. Published online 2020. Accessed March 20, 2023. www.atlasofms.org
3. Lublin FD, Reingold SC, Cohen JA, et al. *VIEWS & REVIEWS Defining the Clinical Course of Multiple Sclerosis The 2013 Revisions*.; 2014.
4. Frischer JM, Bramow S, Dal-Bianco A, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain*. 2009;132(5):1175. doi:10.1093/BRAIN/AWP070
5. Kurtzke JF. Multiple sclerosis in time and space--geographic clues to cause. *J Neurovirol*. 2000;6 Suppl 2:S134-40.
6. Koch-Henriksen N, Sørensen PS. The changing demographic pattern of multiple sclerosis epidemiology. *Lancet Neurol*. 2010;9(5):520-532. doi:10.1016/S1474-4422(10)70064-8
7. Alonso A, Hernán MA. Temporal trends in the incidence of multiple sclerosis A systematic review. Published online 2008.
8. Orton SM, Herrera BM, Yee IM, et al. Sex ratio of multiple sclerosis in Canada: a longitudinal study. *Lancet Neurol*. 2006;5(11):932-936. doi:10.1016/S1474-4422(06)70581-6
9. Yeshokumar AK, Narula S, Banwell B. Pediatric multiple sclerosis. *Curr Opin Neurol*. 2017;30(3):216-221. doi:10.1097/WCO.0000000000000452
10. Bove RM, Healy B, Augustine A, Musallam A, Gholipour T, Chitnis T. Effect of gender on late-onset multiple sclerosis. *Mult Scler*. 2012;18(10):1472-1479. doi:10.1177/1352458512438236
11. Ruggieri M, Iannetti P, Polizzi A, Pavone L, Grimaldi* LME. Multiple sclerosis in children under 10 years of age. *Neurological Sciences*. 2004;25(S4):s326-s335. doi:10.1007/s10072-004-0335-z
12. Klein SL, Flanagan KL. Sex differences in immune responses. *Nature Reviews Immunology 2016 16:10*. 2016;16(10):626-638. doi:10.1038/nri.2016.90
13. Białek M, Zaremba P, Borowicz KK, Czuczwar SJ. Neuroprotective role of testosterone in the nervous system. *Pol J Pharmacol*. 2004;56(5):509-518.
14. Ramien C, Taenzer A, Lupu A, et al. Sex effects on inflammatory and neurodegenerative processes in multiple sclerosis. *Neurosci Biobehav Rev*. 2016;67:137-146. doi:10.1016/J.NEUBIOREV.2015.12.015
15. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. *Nat Rev Neurol*. 2017;13(1):25-36. doi:10.1038/nrneurol.2016.187
16. Sawcer S, Franklin RJM, Ban M. Multiple sclerosis genetics. *Lancet Neurol*. 2014;13(7):700-709. doi:10.1016/S1474-4422(14)70041-9
17. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*. 2011;476(7359):214-219. doi:10.1038/nature10251
18. Baranzini SE, Wang J, Gibson RA, et al. Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. *Hum Mol Genet*. 2009;18(4):767-778. doi:10.1093/hmg/ddn388

19. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: The role of infection. *Ann Neurol*. 2007;61(4):288-299. doi:10.1002/ana.21117
20. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. *Ann Neurol*. 2007;61(6):504-513. doi:10.1002/ana.21141
21. Soldan SS, Lieberman PM. Epstein–Barr virus and multiple sclerosis. *Nature Reviews Microbiology 2022 21:1*. 2022;21(1):51-64. doi:10.1038/s41579-022-00770-5
22. Bjornevik K, Cortese M, Healy BC, et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. *Science (1979)*. 2022;375(6578):296-301. doi:10.1126/science.abj8222
23. Simpson S, Blizzard L, Otahal P, Van der Mei I, Taylor B. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. *J Neurol Neurosurg Psychiatry*. 2011;82(10):1132-1141. doi:10.1136/jnnp.2011.240432
24. Pierrot-Deseilligny C, Souberbielle JC. Vitamin D and multiple sclerosis: An update. *Mult Scler Relat Disord*. 2017;14:35-45. doi:10.1016/j.msard.2017.03.014
25. Zalc B. One hundred and fifty years ago Charcot reported multiple sclerosis as a new neurological disease. *Brain*. 2018;141(12):3482-3488. doi:10.1093/brain/awy287
26. Sormani MP, Rovaris M, Comi G, Filippi M. A reassessment of the plateauing relationship between T2 lesion load and disability in MS. *Neurology*. 2009;73(19):1538-1542. doi:10.1212/WNL.0B013E3181C06679
27. Rocca MA, Amato MP, De Stefano N, et al. Clinical and imaging assessment of cognitive dysfunction in multiple sclerosis. *Lancet Neurol*. 2015;14(3):302-317. doi:10.1016/S1474-4422(14)70250-9
28. Bø L, Vedeler CA, Nyland H, Trapp BD, Mørk SJ. Intracortical multiple sclerosis lesions are not associated with increased lymphocyte infiltration. *Multiple Sclerosis Journal*. 2003;9(4):323-331. doi:10.1191/1352458503ms917oa
29. Peterson JW, Bö L, Mörk S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Ann Neurol*. 2001;50(3):389-400. doi:10.1002/ana.1123
30. Van Der Valk P, De Groot CJA. Staging of multiple sclerosis (MS) lesions: pathology of the time frame of MS. *Neuropathol Appl Neurobiol*. 2000;26(1):2-10. doi:10.1046/j.1365-2990.2000.00217.x
31. Bö L, Mörk S, Kong PA, Nyland H, Pardo CA, Trapp BD. Detection of MHC class II-antigens on macrophages and microglia, but not on astrocytes and endothelia in active multiple sclerosis lesions. *J Neuroimmunol*. 1994;51(2):135-146. doi:10.1016/0165-5728(94)90075-2
32. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal Transection in the Lesions of Multiple Sclerosis. *New England Journal of Medicine*. 1998;338(5):278-285. doi:10.1056/NEJM199801293380502
33. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol*. 2000;47(6):707-717. doi:10.1002/1531-8249(200006)47:6<707::aid-ana3>3.0.co;2-q

34. Metz I, Weigand SD, Popescu BFG, et al. Pathologic heterogeneity persists in early active multiple sclerosis lesions. *Ann Neurol*. 2014;75(5):728-738. doi:10.1002/ana.24163
35. Reich DS, Lucchinetti CF, Calabresi PA. Multiple Sclerosis. *New England Journal of Medicine*. 2018;378(2):169-180. doi:10.1056/NEJMra1401483
36. Prineas JW, Barnard RO, Kwon EE, Sharer LR, Cho ES. Multiple sclerosis: Remyelination of nascent lesions: Remyelination of nascent lesions. *Ann Neurol*. 1993;33(2):137-151. doi:10.1002/ana.410330203
37. Albert M, Antel J, Brück W, Stadelmann C. Extensive Cortical Remyelination in Patients with Chronic Multiple Sclerosis. *Brain Pathology*. 2007;17(2):129-138. doi:10.1111/J.1750-3639.2006.00043.X
38. Franklin RJM, ffrench-Constant C. Remyelination in the CNS: from biology to therapy. *Nat Rev Neurosci*. 2008;9(11):839-855. doi:10.1038/nrn2480
39. Kutzelnigg A, Lucchinetti CF, Stadelmann C, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain*. 2005;128(Pt 11):2705-2712. doi:10.1093/BRAIN/AWH641
40. Lucchinetti CF, Popescu BFG, Bunyan RF, et al. Inflammatory Cortical Demyelination in Early Multiple Sclerosis. *New England Journal of Medicine*. 2011;365(23):2188-2197. doi:10.1056/NEJMoa1100648
41. De Stefano N, Matthews PM, Filippi M, et al. Evidence of early cortical atrophy in MS: relevance to white matter changes and disability. *Neurology*. 2003;60(7):1157-1162. doi:10.1212/01.WNL.0000055926.69643.03
42. Calabrese M, De Stefano N, Atzori M, et al. Detection of Cortical Inflammatory Lesions by Double Inversion Recovery Magnetic Resonance Imaging in Patients With Multiple Sclerosis. *Arch Neurol*. 2007;64(10):1416. doi:10.1001/archneur.64.10.1416
43. Calabrese M, Gallo P. Magnetic resonance evidence of cortical onset of multiple sclerosis. *Multiple Sclerosis Journal*. 2009;15(8):933-941. doi:10.1177/1352458509106510
44. Magliozzi R, Reynolds R, Calabrese M. MRI of cortical lesions and its use in studying their role in MS pathogenesis and disease course. *Brain Pathology*. 2018;28(5):735-742. doi:10.1111/bpa.12642
45. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol*. 2015;15(9):545-558. doi:10.1038/nri3871
46. Lassmann H. Mechanisms of white matter damage in multiple sclerosis. *Glia*. 2014;62(11):1816-1830. doi:10.1002/glia.22597
47. Jelcic I, Al Nimer F, Wang J, et al. Memory B Cells Activate Brain-Homing, Autoreactive CD4+ T Cells in Multiple Sclerosis. *Cell*. 2018;175(1):85-100.e23. doi:10.1016/J.CELL.2018.08.011
48. Cao Y, Goods BA, Raddassi K, et al. Functional inflammatory profiles distinguish myelin-reactive T cells from patients with multiple sclerosis. *Sci Transl Med*. 2015;7(287). doi:10.1126/scitranslmed.aaa8038
49. Alvarez JI, Dodelet-Devillers A, Kebir H, et al. The Hedgehog Pathway Promotes Blood-Brain Barrier Integrity and CNS Immune Quiescence. *Science (1979)*. 2011;334(6063):1727-1731. doi:10.1126/science.1206936
50. Alvarez JI, Saint-Laurent O, Godschalk A, et al. Focal disturbances in the blood–brain barrier are associated with formation of neuroinflammatory lesions. *Neurobiol Dis*. 2015;74:14-24. doi:10.1016/j.nbd.2014.09.016

51. Michel L, Touil H, Pikor NB, Gommerman JL, Prat A, Bar-Or A. B Cells in the Multiple Sclerosis Central Nervous System: Trafficking and Contribution to CNS-Compartmentalized Inflammation. *Front Immunol*. 2015;6. doi:10.3389/fimmu.2015.00636
52. Hauser SL, Bar-Or A, Comi G, et al. Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis. *New England Journal of Medicine*. 2017;376(3):221-234. doi:10.1056/NEJMoa1601277
53. Sergott RC, Bennett JL, Rieckmann P, et al. ATON: Results from a Phase II randomized trial of the B-cell-targeting agent atacicept in patients with optic neuritis. *J Neurol Sci*. 2015;351(1-2):174-178. doi:10.1016/j.jns.2015.02.019
54. Mishra MK, Yong VW. Myeloid cells — targets of medication in multiple sclerosis. *Nat Rev Neurol*. 2016;12(9):539-551. doi:10.1038/nrneurol.2016.110
55. Prinz M, Priller J, Sisodia SS, Ransohoff RM. Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat Neurosci*. 2011;14(10):1227-1235. doi:10.1038/nn.2923
56. van der Valk P, Amor S. Preactive lesions in multiple sclerosis. *Curr Opin Neurol*. 2009;22(3):207-213. doi:10.1097/WCO.0b013e32832b4c76
57. Maggi P, Macri SMC, Gaitán MI, et al. The formation of inflammatory demyelinated lesions in cerebral white matter. *Ann Neurol*. 2014;76(4):594-608. doi:10.1002/ana.24242
58. Butovsky O, Jedrychowski MP, Moore CS, et al. Erratum: Corrigendum: Identification of a unique TGF-β–dependent molecular and functional signature in microglia. *Nat Neurosci*. 2014;17(9):1286-1286. doi:10.1038/nn0914-1286d
59. Kaskow BJ, Baecher-Allan C. Effector T Cells in Multiple Sclerosis. *Cold Spring Harb Perspect Med*. 2018;8(4):a029025. doi:10.1101/cshperspect.a029025
60. Gold R, Hartung HP, Lassmann H. T-cell apoptosis in autoimmune diseases: termination of inflammation in the nervous system and other sites with specialized immune-defense mechanisms. *Trends Neurosci*. 1997;20(9):399-404. doi:10.1016/S0166-2236(97)01079-5
61. Miller DH, Chard DT, Ciccarelli O. Clinically isolated syndromes. *Lancet Neurol*. 2012;11(2):157-169. doi:10.1016/S1474-4422(11)70274-5
62. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. *Ann Neurol*. 2011;69(2):292-302. doi:10.1002/ANA.22366
63. Woung LC, Peng PH, Liu CC, et al. A Nine-Year Population-Based Cohort Study on the Risk of Multiple Sclerosis in Patients with Optic Neuritis. *Tohoku J Exp Med*. 2013;231(3):171-177. doi:10.1620/TJEM.231.171
64. Kuhle J, Disanto G, Dobson R, et al. Conversion from clinically isolated syndrome to multiple sclerosis: A large multicantre study. *Multiple Sclerosis*. 2015;21(8):1013-1024. doi:10.1177/1352458514568827/ASSET/IMAGES/LARGE/10.1177_1352458514568827-FIG1.JPEG
65. Brownlee WJ, Hardy TA, Fazekas F, Miller DH. Diagnosis of multiple sclerosis: progress and challenges. *The Lancet*. 2017;389(10076):1336-1346. doi:10.1016/S0140-6736(16)30959-X
66. Pardini M, Uccelli A, Grafman J, Yaldizli Ö, Mancardi G, Roccatagliata L. Isolated cognitive relapses in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2014;85(9):1035-1037. doi:10.1136/JNNP-2013-307275

67. Zipoli V, Goretti B, Hakiki B, et al. Cognitive impairment predicts conversion to multiple sclerosis in clinically isolated syndromes. *Multiple Sclerosis Journal*. 2010;16(1):62-67. doi:10.1177/1352458509350311
68. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). *Neurology*. 1983;33(11):1444-1444. doi:10.1212/WNL.33.11.1444
69. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol*. 2018;17(2):162-173. doi:10.1016/S1474-4422(17)30470-2
70. Nelson F, Poonawalla AH, Hou P, Huang F, Wolinsky JS, Narayana PA. Improved Identification of Intracortical Lesions in Multiple Sclerosis with Phase-Sensitive Inversion Recovery in Combination with Fast Double Inversion Recovery MR Imaging. *American Journal of Neuroradiology*. 2007;28(9):1645-1649. doi:10.3174/AJNR.A0645
71. De Stefano N, Giorgio A, Tintoré M, et al. Radiologically isolated syndrome or subclinical multiple sclerosis: MAGNIMS consensus recommendations. *Multiple Sclerosis Journal*. 2018;24(2):214-221. doi:10.1177/1352458517717808
72. Okuda DT, Siva A, Kantarci O, et al. Radiologically Isolated Syndrome: 5-Year Risk for an Initial Clinical Event. *PLoS One*. 2014;9(3):e90509. doi:10.1371/journal.pone.0090509
73. Bloomgren G, Richman S, Hotermans C, et al. Risk of Natalizumab-Associated Progressive Multifocal Leukoencephalopathy. *New England Journal of Medicine*. 2012;366(20):1870-1880. doi:10.1056/NEJMoa1107829
74. McGinley MP, Goldschmidt CH, Rae-Grant AD. Diagnosis and Treatment of Multiple Sclerosis. *JAMA*. 2021;325(8):765. doi:10.1001/jama.2020.26858
75. Hauser SL, Cree BAC. Treatment of Multiple Sclerosis: A Review. *Am J Med*. 2020;133(12):1380-1390.e2. doi:10.1016/j.amjmed.2020.05.049
76. Lassmann H. Targets of therapy in progressive MS. *Multiple Sclerosis*. 2017;23(12):1593-1599. doi:10.1177/1352458517729455/ASSET/IMAGES/LARGE/10.1177_1352458517729455-FIG2.JPEG
77. Kuhlmann T, Moccia M, Coetzee T, et al. Multiple sclerosis progression: time for a new mechanism-driven framework. *Lancet Neurol*. 2023;22(1):78-88. doi:10.1016/S1474-4422(22)00289-7
78. Charcot JM. Lectures on the Diseases of the Nervous System. *London: New Sydenham Society, 1877*.
79. DeLuca J, Chiaravalloti ND, Sandroff BM. Treatment and management of cognitive dysfunction in patients with multiple sclerosis. *Nature Reviews Neurology 2020 16:6*. 2020;16(6):319-332. doi:10.1038/s41582-020-0355-1
80. Benedict RHB, Amato MP, DeLuca J, Geurts JJG. Cognitive impairment in multiple sclerosis: clinical management, MRI, and therapeutic avenues. *Lancet Neurol*. 2020;19(10):860-871. doi:10.1016/S1474-4422(20)30277-5
81. Ruano L, Portaccio E, Goretti B, et al. Age and disability drive cognitive impairment in multiple sclerosis across disease subtypes. *Multiple Sclerosis Journal*. 2017;23(9):1258-1267. doi:10.1177/1352458516674367
82. Amato MP, Razzolini L, Goretti B, et al. Cognitive reserve and cortical atrophy in multiple sclerosis: A longitudinal study. *Neurology*. 2013;80(19):1728-1733. doi:10.1212/WNL.0b013e3182918c6f

83. Moccia M, Lanzillo R, Palladino R, et al. Cognitive impairment at diagnosis predicts 10-year multiple sclerosis progression. *Mult Scler*. 2016;22(5):659-667. doi:10.1177/1352458515599075
84. Cavaco S, Ferreira I, Moreira I, et al. Cognitive dysfunction and mortality in multiple sclerosis: Long-term retrospective review. *Mult Scler*. 2022;28(9):1382-1391. doi:10.1177/13524585211066598
85. De Meo E, Portaccio E, Giorgio A, et al. Identifying the Distinct Cognitive Phenotypes in Multiple Sclerosis. *JAMA Neurol*. 2021;78(4):414-425. doi:10.1001/JAMANEUROL.2020.4920
86. Lin XG, Zhang XL, Liu QQ, et al. Social cognition in multiple sclerosis and its subtypes: A meta-analysis. *Mult Scler Relat Disord*. 2021;52. doi:10.1016/J.MSARD.2021.102973
87. Radlak B, Cooper C, Summers F, Phillips LH. Multiple sclerosis, emotion perception and social functioning. *J Neuropsychol*. 2021;15(3):500-515. doi:10.1111/JNP.12237
88. Portaccio E, De Meo E, Bellinvia A, Amato MP. Cognitive Issues in Pediatric Multiple Sclerosis. *Brain Sci*. 2021;11(4). doi:10.3390/BRAINSCI11040442
89. Amato MP, Krupp LB, Charvet LE, Penner I, Till C. Pediatric multiple sclerosis: Cognition and mood. *Neurology*. 2016;87(9 Suppl 2):S82-S87. doi:10.1212/WNL.0000000000002883
90. Portaccio E, Bellinvia A, Razzolini L, et al. Long-term Cognitive Outcomes and Socioprofessional Attainment in People With Multiple Sclerosis With Childhood Onset. *Neurology*. 2022;98(16):e1626-e1636. doi:10.1212/WNL.0000000000200115
91. Portaccio E, Bellinvia A, Razzolini L, et al. Long-term Cognitive Outcomes and Socioprofessional Attainment in People With Multiple Sclerosis With Childhood Onset. *Neurology*. 2022;98(16):e1626-e1636. doi:10.1212/WNL.0000000000200115
92. Feinstein A. Mood disorders in multiple sclerosis and the effects on cognition. *J Neurol Sci*. 2006;245(1-2):63-66. doi:10.1016/J.JNS.2005.08.020
93. Andreasen AK, Spliid PE, Andersen H, Jakobsen J. Fatigue and processing speed are related in multiple sclerosis. *Eur J Neurol*. 2010;17(2):212-218. doi:10.1111/J.1468-1331.2009.02776.X
94. Sumowski JF, Horng S, Brandstadter R, et al. Sleep disturbance and memory dysfunction in early multiple sclerosis. *Ann Clin Transl Neurol*. 2021;8(6):1172-1182. doi:10.1002/ACN3.51262
95. Oreja-Guevara C, Blanco TA, Ruiz LB, Pérez MÁH, Meca-Lallana V, Ramió-Torrentà L. Cognitive Dysfunctions and Assessments in Multiple Sclerosis. *Front Neurol*. 2019;10(JUN). doi:10.3389/FNEUR.2019.00581
96. Dineen RA, Vilisaar J, Hlinka J, et al. Disconnection as a mechanism for cognitive dysfunction in multiple sclerosis. *Brain*. 2009;132(Pt 1):239-249. doi:10.1093/BRAIN/AWN275
97. Calabrese M, Agosta F, Rinaldi F, et al. Cortical lesions and atrophy associated with cognitive impairment in relapsing-remitting multiple sclerosis. *Arch Neurol*. 2009;66(9):1144-1150. doi:10.1001/ARCHNEUROL.2009.174
98. Harrison DM, Roy S, Oh J, et al. Association of Cortical Lesion Burden on 7-T Magnetic Resonance Imaging With Cognition and Disability in Multiple Sclerosis. *JAMA Neurol*. 2015;72(9):1004. doi:10.1001/jamaneurol.2015.1241

99. Preziosa P, Pagani E, Morelli ME, et al. DT MRI microstructural cortical lesion damage does not explain cognitive impairment in MS. *Multiple Sclerosis Journal*. 2017;23(14):1918-1928. doi:10.1177/1352458516689147
100. Bellmann-Strobl J, Wuerfel J, Aktas O, et al. Poor PASAT performance correlates with MRI contrast enhancement in multiple sclerosis. *Neurology*. 2009;73(20):1624-1627. doi:10.1212/WNL.0b013e3181c1de4f
101. Di Filippo M, Sarchielli P, Picconi B, Calabresi P. Neuroinflammation and synaptic plasticity: theoretical basis for a novel, immune-centred, therapeutic approach to neurological disorders. *Trends Pharmacol Sci*. 2008;29(8):402-412. doi:10.1016/j.tips.2008.06.005
102. Bonnier G, Roche A, Romascano D, et al. Multicontrast MRI Quantification of Focal Inflammation and Degeneration in Multiple Sclerosis. *Biomed Res Int*. 2015;2015:1-9. doi:10.1155/2015/569123
103. Steenwijk MD, Geurts JJG, Daams M, et al. Cortical atrophy patterns in multiple sclerosis are non-random and clinically relevant. *Brain*. 2016;139(1):115-126. doi:10.1093/brain/awv337
104. Eijlers AJC, Dekker I, Steenwijk MD, et al. Cortical atrophy accelerates as cognitive decline worsens in multiple sclerosis. *Neurology*. 2019;93(14):E1348-E1359. doi:10.1212/WNL.0000000000008198
105. Bergsland N, Zivadinov R, Dwyer MG, Weinstock-Guttman B, Benedict RHB. Localized atrophy of the thalamus and slowed cognitive processing speed in MS patients. *Mult Scler*. 2016;22(10):1327-1336. doi:10.1177/1352458515616204
106. Batista S, Zivadinov R, Hoogs M, et al. Basal ganglia, thalamus and neocortical atrophy predicting slowed cognitive processing in multiple sclerosis. *J Neurol*. 2012;259(1):139-146. doi:10.1007/s00415-011-6147-1
107. Planche V, Koubiyr I, Romero JE, et al. Regional hippocampal vulnerability in early multiple sclerosis: Dynamic pathological spreading from dentate gyrus to CA 1. *Hum Brain Mapp*. 2018;39(4):1814-1824. doi:10.1002/hbm.23970
108. Cocozza S, Petracca M, Mormina E, et al. Cerebellar lobule atrophy and disability in progressive MS. *J Neurol Neurosurg Psychiatry*. 2017;88(12):1065-1072. doi:10.1136/jnnp-2017-316448
109. Granberg T, Martola J, Bergendal G, et al. Corpus callosum atrophy is strongly associated with cognitive impairment in multiple sclerosis: Results of a 17-year longitudinal study. *Multiple Sclerosis Journal*. 2015;21(9):1151-1158. doi:10.1177/1352458514560928
110. Batista S, d'Almeida OC, Afonso A, et al. Impairment of social cognition in multiple sclerosis: Amygdala atrophy is the main predictor. *Multiple Sclerosis Journal*. 2017;23(10):1358-1366. doi:10.1177/1352458516680750
111. Meijer KA, Eijlers AJC, Douw L, et al. Increased connectivity of hub networks and cognitive impairment in multiple sclerosis. *Neurology*. 2017;88(22):2107-2114. doi:10.1212/WNL.0000000000003982
112. d'Ambrosio A, Valsasina P, Gallo A, et al. Reduced dynamics of functional connectivity and cognitive impairment in multiple sclerosis. *Mult Scler*. 2020;26(4):476-488. doi:10.1177/1352458519837707
113. Tona F, Petsas N, Sbardella E, et al. Multiple sclerosis: altered thalamic resting-state functional connectivity and its effect on cognitive function. *Radiology*. 2014;271(3):814-821. doi:10.1148/RADIOL.14131688

114. Rocca MA, Valsasina P, Absinta M, et al. Default-mode network dysfunction and cognitive impairment in progressive MS. *Neurology*. 2010;74(16):1252-1259. doi:10.1212/WNL.0B013E3181D9ED91
115. Hawellek DJ, Hipp JF, Lewis CM, Corbetta M, Engel AK. Increased functional connectivity indicates the severity of cognitive impairment in multiple sclerosis. *Proceedings of the National Academy of Sciences*. 2011;108(47):19066-19071. doi:10.1073/pnas.1110024108
116. Schoonheim MM, Broeders TAA, Geurts JJG. The network collapse in multiple sclerosis: An overview of novel concepts to address disease dynamics. *Neuroimage Clin*. 2022;35. doi:10.1016/J.NICL.2022.103108
117. McNicholas N, O'Connell K, Yap SM, Killeen RP, Hutchinson M, McGuigan C. Cognitive dysfunction in early multiple sclerosis: a review. *QJM: An International Journal of Medicine*. 2018;111(6):359-364. doi:10.1093/qjmed/hcx070
118. Guimarães J, Sá MJ. Cognitive dysfunction in multiple sclerosis. *Front Neurol*. 2012;3. doi:10.3389/FNEUR.2012.00074
119. Kinsinger SW, Lattie E, Mohr DC. Relationship between depression, fatigue, subjective cognitive impairment, and objective neuropsychological functioning in patients with multiple sclerosis. *Neuropsychology*. 2010;24(5):573-580. doi:10.1037/A0019222
120. Rao SM, Leo GJ, Bernardin L, Unverzagt F. Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction. *Neurology*. 1991;41(5):685-691. doi:10.1212/WNL.41.5.685
121. Benedict RHB, Deluca J, Phillips G, LaRocca N, Hudson LD, Rudick R. Validity of the Symbol Digit Modalities Test as a cognition performance outcome measure for multiple sclerosis. *Mult Scler*. 2017;23(5):721-733. doi:10.1177/1352458517690821
122. Benedict RHB, Cookfair D, Gavett R, et al. Validity of the minimal assessment of cognitive function in multiple sclerosis (MACFIMS). *J Int Neuropsychol Soc*. 2006;12(4):549-558. doi:10.1017/S1355617706060723
123. Langdon DW, Amato MP, Boringa J, et al. Recommendations for a Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS). *Mult Scler*. 2012;18(6):891-898. doi:10.1177/1352458511431076
124. Wojcik CM, Beier M, Costello K, et al. Computerized neuropsychological assessment devices in multiple sclerosis: A systematic review. *Mult Scler*. 2019;25(14):1848-1869. doi:10.1177/1352458519879094
125. Rao SM, Losinski G, Mourany L, et al. Processing speed test: Validation of a self-administered, iPad®-based tool for screening cognitive dysfunction in a clinic setting. *Mult Scler*. 2017;23(14):1929-1937. doi:10.1177/1352458516688955
126. Kalb R, Beier M, Benedict RHB, et al. Recommendations for cognitive screening and management in multiple sclerosis care. *Mult Scler*. 2018;24(13):1665-1680. doi:10.1177/1352458518803785
127. Benedict RHB, Fishman I, McClellan MM, Bakshi R, Weinstock-Guttman B. Validity of the Beck Depression Inventory-Fast Screen in multiple sclerosis. *Mult Scler*. 2003;9(4):393-396. doi:10.1191/1352458503MS902OA

128. Honarmand K, Feinstein A. Validation of the Hospital Anxiety and Depression Scale for use with multiple sclerosis patients. *Mult Scler*. 2009;15(12):1518-1524. doi:10.1177/1352458509347150
129. Ottonello M, Pellicciari L, Giordano A, Foti C. Rasch analysis of the Fatigue Severity Scale in Italian subjects with multiple sclerosis. *J Rehabil Med*. 2016;48(7):597-603. doi:10.2340/16501977-2116
130. Chen MH, Chiaravalloti ND, DeLuca J. Neurological update: cognitive rehabilitation in multiple sclerosis. *J Neurol*. 2021;268(12):4908-4914. doi:10.1007/s00415-021-10618-2
131. Naeeni Davarani M, Arian Darestani A, Hassani-Abharian P, Vaseghi S, Zarrindast MR, Nasehi M. RehaCom rehabilitation training improves a wide-range of cognitive functions in multiple sclerosis patients. *Appl Neuropsychol Adult*. 2022;29(2):262-272. doi:10.1080/23279095.2020.1747070
132. Chiaravalloti ND, Moore NB, Nikelshpur OM, DeLuca J. An RCT to treat learning impairment in multiple sclerosis: The MEMREHAB trial. *Neurology*. 2013;81(24):2066-2072. doi:10.1212/01.WNL.0000437295.97946.A8
133. Landmeyer NC, Bürkner PC, Wiendl H, et al. Disease-modifying treatments and cognition in relapsing-remitting multiple sclerosis: A meta-analysis. *Neurology*. 2020;94(22):E2373-E2383. doi:10.1212/WNL.0000000000009522
134. Chen MH, Goverover Y, Genova HM, DeLuca J. Cognitive Efficacy of Pharmacologic Treatments in Multiple Sclerosis: A Systematic Review. *CNS Drugs*. 2020;34(6):599-628. doi:10.1007/S40263-020-00734-4
135. DeLuca J, Schippling S, Montalban X, et al. Effect of Ozanimod on Symbol Digit Modalities Test Performance in Relapsing MS. *Mult Scler Relat Disord*. 2021;48. doi:10.1016/J.MSARD.2020.102673
136. Cree BAC, Arnold DL, Fox RJ, et al. Long-term efficacy and safety of siponimod in patients with secondary progressive multiple sclerosis: Analysis of EXPAND core and extension data up to >5 years. *Mult Scler*. 2022;28(10):1591-1605. doi:10.1177/13524585221083194
137. Chen MH, Goverover Y, Genova HM, Deluca J. Cognitive Efficacy of Pharmacologic Treatments in Multiple Sclerosis: A Systematic Review. *CNS Drugs*. 123AD;34. doi:10.1007/s40263-020-00734-4
138. Cotter J, Muhlert N, Talwar A, Granger K. Examining the effectiveness of acetylcholinesterase inhibitors and stimulant-based medications for cognitive dysfunction in multiple sclerosis: A systematic review and meta-analysis. *Neurosci Biobehav Rev*. 2018;86:99-107. doi:10.1016/J.NEUBIOREV.2018.01.006
139. Turalde CWR, Espiritu AI, Anlacan VMM. Memantine for Multiple Sclerosis: A Systematic Review and Meta-Analysis of Randomized Trials. *Front Neurol*. 2020;11:574748. doi:10.3389/FNEUR.2020.574748
140. Zhang E, Tian X, Li R, et al. Dalfampridine in the treatment of multiple sclerosis: a meta-analysis of randomised controlled trials. *Orphanet J Rare Dis*. 2021;16(1). doi:10.1186/S13023-021-01694-8
141. Ghielen I, Rutten S, Boeschoten RE, et al. The effects of cognitive behavioral and mindfulness-based therapies on psychological distress in patients with multiple sclerosis, Parkinson's disease and Huntington's disease: Two meta-analyses. *J Psychosom Res*. 2019;122:43-51. doi:10.1016/J.JPSYCHORES.2019.05.001

142. Zarotti N, Eccles F, Broyd A, Longinotti C, Mobley A, Simpson J. Third wave cognitive behavioural therapies for people with multiple sclerosis: a scoping review. Published online 2022. doi:10.1080/09638288.2022.2069292
143. Koziol LF, Budding D, Andreasen N, et al. Consensus Paper: The Cerebellum's Role in Movement and Cognition. *The Cerebellum*. 2014;13(1):151-177. doi:10.1007/s12311-013-0511-x
144. Stoodley CJ, Schmahmann JD. Functional topography of the human cerebellum. In: ; 2018:59-70. doi:10.1016/B978-0-444-63956-1.00004-7
145. Crick FC, Koch C. What is the function of the claustrum? *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2005;360(1458):1271-1279. doi:10.1098/RSTB.2005.1661
146. Mancall, E.L., Brock, D.G., 2011a. Gray's Clinical Neuroanatomy: The Anatomic Basis for Clinical Neuroscience, 1st ed. Elsevier Health Sciences, Philadelphia, PA, USA, pp. 229–244. Chapter 13, Cerebellum. .
147. Stoodley CJ, Schmahmann JD. Functional topography of the human cerebellum. In: ; 2018:59-70. doi:10.1016/B978-0-444-63956-1.00004-7
148. Schmahmann JD. The cerebellum and cognition. *Neurosci Lett*. 2019;688:62-75. doi:10.1016/J.NEULET.2018.07.005
149. Schmahmann JD. From movement to thought: Anatomic substrates of the cerebellar contribution to cognitive processing. *Hum Brain Mapp*. 1996;4(3):174-198. doi:10.1002/(SICI)1097-0193(1996)4:3<174::AID-HBM3>3.0.CO;2-0
150. Stoodley C, Schmahmann J. Functional topography in the human cerebellum: A meta-analysis of neuroimaging studies. *Neuroimage*. 2009;44(2):489-501. doi:10.1016/j.neuroimage.2008.08.039
151. Strick PL, Dum RP, Fiez JA. Cerebellum and nonmotor function. *Annu Rev Neurosci*. 2009;32:413-434. doi:10.1146/ANNUREV.NEURO.31.060407.125606
152. Diedrichsen J, Balsters JH, Flavell J, Cussans E, Ramnani N. A probabilistic MR atlas of the human cerebellum. *Neuroimage*. 2009;46(1):39-46. doi:10.1016/J.NEUROIMAGE.2009.01.045
153. Schmahmann, J. D., Doyon, J., Petrides, M., Evans, A. C., & Toga, A. W. (2000). MRI atlas of the human cerebellum. Academic press.
154. Devita M, Alberti F, Fagnani M, et al. Novel insights into the relationship between cerebellum and dementia: A narrative review as a toolkit for clinicians. *Ageing Res Rev*. 2021;70:101389. doi:10.1016/j.arr.2021.101389
155. Schmahmann JD, Pandya DN. The Cerebrocerebellar System. In: ; 1997:31-60. doi:10.1016/S0074-7742(08)60346-3
156. D'Ambrosio A, Pagani E, Riccitelli GC, et al. Cerebellar contribution to motor and cognitive performance in multiple sclerosis: An MRI sub-regional volumetric analysis. *http://dx.doi.org/101177/1352458516674567*. 2016;23(9):1194-1203. doi:10.1177/1352458516674567
157. Basser PJ, Mattiello J, LeBihan D. MR diffusion tensor spectroscopy and imaging. *Biophys J*. 1994;66(1):259-267. doi:10.1016/S0006-3495(94)80775-1
158. Friston KJ. Functional and effective connectivity in neuroimaging: A synthesis. *Hum Brain Mapp*. 1994;2(1-2):56-78. doi:10.1002/hbm.460020107
159. Biswal B, Zerrin Yetkin F, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar mri. *Magn Reson Med*. 1995;34(4):537-541. doi:10.1002/MRM.1910340409

160. Ogawa S, Tank DW, Menon R, et al. Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci U S A*. 1992;89(13):5951-5955. doi:10.1073/PNAS.89.13.5951
161. Zhang S, Li X, Lv J, Jiang X, Guo L, Liu T. Characterizing and differentiating task-based and resting state fMRI signals via two-stage sparse representations. doi:10.1007/s11682-015-9359-7
162. Lee MH, Smyser CD, Shimony JS. Resting-State fMRI: A Review of Methods and Clinical Applications. *American Journal of Neuroradiology*. 2013;34(10):1866-1872. doi:10.3174/ajnr.A3263
163. Habas C. Functional Connectivity of the Cognitive Cerebellum. *Front Syst Neurosci*. 2021;15. doi:10.3389/fnsys.2021.642225
164. Rolls ET, Huang CC, Lin CP, Feng J, Joliot M. Automated anatomical labelling atlas 3. *Neuroimage*. 2020;206:116189. doi:10.1016/j.neuroimage.2019.116189
165. Schaefer A, Kong R, Gordon EM, et al. Local-Global Parcellation of the Human Cerebral Cortex from Intrinsic Functional Connectivity MRI. *Cereb Cortex*. 2018;28(9):3095-3114. doi:10.1093/CERCOR/BHX179
166. Beckmann CF, DeLuca M, Devlin JT, Smith SM. Investigations into resting-state connectivity using independent component analysis. *Philos Trans R Soc Lond B Biol Sci*. 2005;360(1457):1001-1013. doi:10.1098/RSTB.2005.1634
167. Thomas Yeo BT, Krienen FM, Sepulcre J, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J Neurophysiol*. 2011;106(3):1125-1165. doi:10.1152/jn.00338.2011
168. Buckner RL, Krienen FM, Castellanos A, Diaz JC, Thomas Yeo BT. The organization of the human cerebellum estimated by intrinsic functional connectivity. *J Neurophysiol*. 2011;106(5):2322-2345. doi:10.1152/JN.00339.2011
169. O'Reilly JX, Beckmann CF, Tomassini V, Ramnani N, Johansen-Berg H. Distinct and overlapping functional zones in the cerebellum defined by resting state functional connectivity. *Cereb Cortex*. 2010;20(4):953-965. doi:10.1093/CERCOR/BHP157
170. Krienen FM, Buckner RL. Segregated fronto-cerebellar circuits revealed by intrinsic functional connectivity. *Cereb Cortex*. 2009;19(10):2485-2497. doi:10.1093/CERCOR/BHP135
171. Habas C, Kamdar N, Nguyen D, et al. Distinct cerebellar contributions to intrinsic connectivity networks. *J Neurosci*. 2009;29(26):8586-8594. doi:10.1523/JNEUROSCI.1868-09.2009
172. Bernard JA, Peltier SJ, Benson BL, et al. Dissociable Functional Networks of the Human Dentate Nucleus. *Cerebral Cortex*. 2014;24(8):2151-2159. doi:10.1093/CERCOR/BHT065
173. Buckner RL, Krienen FM, Castellanos A, Diaz JC, Thomas Yeo BT. The organization of the human cerebellum estimated by intrinsic functional connectivity. *J Neurophysiol*. 2011;106(5):2322-2345. doi:10.1152/JN.00339.2011
174. E KH, Chen SHA, Ho MHR, Desmond JE. A meta-analysis of cerebellar contributions to higher cognition from PET and fMRI studies. *Hum Brain Mapp*. 2014;35(2):593-615. doi:10.1002/hbm.22194

175. Guell X, Gabrieli JDE, Schmahmann JD. Triple representation of language, working memory, social and emotion processing in the cerebellum: convergent evidence from task and seed-based resting-state fMRI analyses in a single large cohort. *Neuroimage*. 2018;172:437-449. doi:10.1016/J.NEUROIMAGE.2018.01.082
176. Guell X, Schmahmann JD, Gabrieli JDE, Ghosh SS. Functional gradients of the cerebellum. *Elife*. 2018;7. doi:10.7554/ELIFE.36652
177. Katsumi Y, Zhang J, Chen D, et al. Correspondence of functional connectivity gradients across human isocortex, cerebellum, and hippocampus. *Commun Biol*. 2023;6(1):401. doi:10.1038/s42003-023-04796-0
178. Hohenfeld C, Werner CJ, Reetz K. Resting-state connectivity in neurodegenerative disorders: Is there potential for an imaging biomarker? *Neuroimage Clin*. 2018;18:849-870. doi:10.1016/j.nicl.2018.03.013
179. Stoodley CJ, Limperopoulos C. Structure-function relationships in the developing cerebellum: Evidence from early-life cerebellar injury and neurodevelopmental disorders. *Semin Fetal Neonatal Med*. 2016;21(5):356-364. doi:10.1016/J.SINY.2016.04.010
180. Schmahmann JD, Macmore J, Vangel M. Cerebellar stroke without motor deficit: Clinical evidence for motor and non-motor domains within the human cerebellum. *Neuroscience*. 2009;162(3):852-861. doi:10.1016/j.neuroscience.2009.06.023
181. Stoodley CJ, MacMore JP, Makris N, Sherman JC, Schmahmann JD. Location of lesion determines motor vs. cognitive consequences in patients with cerebellar stroke. *Neuroimage Clin*. 2016;12:765-775. doi:10.1016/J.NICL.2016.10.013
182. Holmes G. The cerebellum of man. *Brain*. 1939;62(1):1-30. doi:10.1093/BRAIN/62.1.1
183. Kase CS, Norrving B, Levine SR, et al. Cerebellar infarction. Clinical and anatomic observations in 66 cases. *Stroke*. 1993;24(1):76-83. doi:10.1161/01.STR.24.1.76
184. Schoch B, Dimitrova A, Gizewski ER, Timmann D. Functional localization in the human cerebellum based on voxelwise statistical analysis: a study of 90 patients. *Neuroimage*. 2006;30(1):36-51. doi:10.1016/J.NEUROIMAGE.2005.09.018
185. Kansal K, Yang Z, Fishman AM, et al. Structural cerebellar correlates of cognitive and motor dysfunctions in cerebellar degeneration. *Brain*. 2017;140(3):707. doi:10.1093/BRAIN/AWW327
186. D'Ambrosio A, Pagani E, Riccitelli GC, et al. Cerebellar contribution to motor and cognitive performance in multiple sclerosis: An MRI sub-regional volumetric analysis. *Mult Scler*. 2017;23(9):1194-1203. doi:10.1177/1352458516674567
187. Konczak J, Schoch B, Dimitrova A, Gizewski E, Timmann D. Functional recovery of children and adolescents after cerebellar tumour resection. *Brain*. 2005;128(6):1428-1441. doi:10.1093/BRAIN/AWH385
188. Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. *Brain*. 1998;121 (Pt 4)(4):561-579. doi:10.1093/BRAIN/121.4.561
189. Levisohn L, Cronin-Golomb A, Schmahmann JD. Neuropsychological consequences of cerebellar tumour resection in children: cerebellar cognitive affective syndrome in a paediatric population. *Brain*. 2000;123 (Pt 5)(5):1041-1050. doi:10.1093/BRAIN/123.5.1041

190. Hoche F, Guell X, Vangel MG, Sherman JC, Schmahmann JD. The cerebellar cognitive affective/Schmahmann syndrome scale. *Brain*. 2018;141(1):248-270. doi:10.1093/brain/awx317
191. Schmahmann JD. From movement to thought: Anatomic substrates of the cerebellar contribution to cognitive processing. *Hum Brain Mapp*. 1996;4(3):174-198. doi:10.1002/(SICI)1097-0193(1996)4:3<174::AID-HBM3>3.0.CO;2-0
192. Diedrichsen J, King M, Hernandez-Castillo C, Sereno M, Ivry RB. Universal Transform or Multiple Functionality? Understanding the Contribution of the Human Cerebellum across Task Domains. *Neuron*. 2019;102(5):918-928. doi:10.1016/j.neuron.2019.04.021
193. Marr D. A theory of cerebellar cortex. *J Physiol*. 1969;202(2):437. doi:10.1113/JPHYSIOL.1969.SP008820
194. Wilkins A. Cerebellar Dysfunction in Multiple Sclerosis. *Front Neurol*. 2017;8. doi:10.3389/fneur.2017.00312
195. D'Ambrosio A, Pagani E, Riccitelli GC, et al. Cerebellar contribution to motor and cognitive performance in multiple sclerosis: An MRI sub-regional volumetric analysis. *Multiple Sclerosis Journal*. 2017;23(9):1194-1203. doi:10.1177/1352458516674567
196. Tornes L, Conway B, Sheremata W. Multiple Sclerosis and the Cerebellum. *Neurol Clin*. 2014;32(4):957-977. doi:10.1016/j.ncl.2014.08.001
197. Boonstra F, Florescu G, Evans A, et al. Tremor in multiple sclerosis is associated with cerebello-thalamic pathology. *J Neural Transm*. 2017;124(12):1509-1514. doi:10.1007/s00702-017-1798-4
198. Koch M, Mostert J, Heersema D, De Keyser J. Tremor in multiple sclerosis. *J Neurol*. 2007;254(2):133-145. doi:10.1007/s00415-006-0296-7
199. Lazzarotto A, Margoni M, Franciotta S, et al. Selective Cerebellar Atrophy Associates with Depression and Fatigue in the Early Phases of Relapse-Onset Multiple Sclerosis. *The Cerebellum*. 2020;19(2):192-200. doi:10.1007/s12311-019-01096-4
200. Weier K, Penner IK, Magon S, et al. Cerebellar Abnormalities Contribute to Disability Including Cognitive Impairment in Multiple Sclerosis. *PLoS One*. 2014;9(1):e86916. doi:10.1371/journal.pone.0086916
201. Weier K, Till C, Fonov V, et al. Contribution of the cerebellum to cognitive performance in children and adolescents with multiple sclerosis. *Multiple Sclerosis Journal*. 2016;22(5):599-607. doi:10.1177/1352458515595132
202. Archibald CJ, Wei X, Scott JN, et al. Posterior fossa lesion volume and slowed information processing in multiple sclerosis. *Brain*. 2004;127(7):1526-1534. doi:10.1093/brain/awh167
203. Barletta VT, Herranz E, Treaba CA, et al. Evidence of diffuse cerebellar neuroinflammation in multiple sclerosis by ^{11}C-PBR28 MR-PET. *Multiple Sclerosis Journal*. 2020;26(6):668-678. doi:10.1177/1352458519843048
204. Granberg T, Fan Q, Treaba CA, et al. In vivo characterization of cortical and white matter neuroaxonal pathology in early multiple sclerosis. *Brain*. 2017;140(11):2912-2926. doi:10.1093/brain/awx247
205. Savini G, Pardini M, Castellazzi G, et al. Default Mode Network Structural Integrity and Cerebellar Connectivity Predict Information Processing Speed Deficit in Multiple Sclerosis. *Front Cell Neurosci*. 2019;13. doi:10.3389/fncel.2019.00021

206. Hoche F, Guell X, Vangel MG, Sherman JC, Schmahmann JD. The cerebellar cognitive affective/Schmahmann syndrome scale. *Brain*. 2018;141(1):248-270. doi:10.1093/brain/awx317
207. Goretti B, Niccolai C, Hakiki B, et al. The brief international cognitive assessment for multiple sclerosis (BICAMS): normative values with gender, age and education corrections in the Italian population. *BMC Neurol*. 2014;14(1):171. doi:10.1186/s12883-014-0171-6
208. Nocentini U, Giordano A, Di Vincenzo S, Panella M, Pasqualetti P. The Symbol Digit Modalities Test - Oral version: Italian normative data. *Funct Neurol*. 2006;21(2):93-96.
209. Mattioli F, Stampatori C, Bellomi F, et al. Assessing executive function with the D-KEFS sorting test: normative data for a sample of the Italian adult population. *Neurological Sciences*. 2014;35(12):1895-1902. doi:10.1007/s10072-014-1857-7
210. Beck AT, Steer RA, Ball R, Ranieri WF. Comparison of Beck Depression Inventories-IA and-II in Psychiatric Outpatients. *J Pers Assess*. 1996;67(3):588-597. doi:10.1207/s15327752jpa6703_13
211. Ottonello M, Pellicciari L, Giordano A, Foti C. Rasch analysis of the Fatigue Severity Scale in Italian subjects with multiple sclerosis. *J Rehabil Med*. 2016;48(7):597-603. doi:10.2340/16501977-2116
212. Abderrakib A, Ligot N, Naeije G. Cerebellar cognitive affective syndrome after acute cerebellar stroke. *Front Neurol*. 2022;13. doi:10.3389/fneur.2022.906293
213. Thieme A, Faber J, Sulzer P, et al. The CCAS-scale in hereditary ataxias: helpful on the group level, particularly in SCA3, but limited in individual patients. *J Neurol*. 2022;269(8):4363-4374. doi:10.1007/s00415-022-11071-5
214. Hawellek DJ, Hipp JF, Lewis CM, Corbetta M, Engel AK. Increased functional connectivity indicates the severity of cognitive impairment in multiple sclerosis. *Proceedings of the National Academy of Sciences*. 2011;108(47):19066-19071. doi:10.1073/pnas.1110024108
215. Has Silemek AC, Fischer L, Pöttgen J, et al. Functional and structural connectivity substrates of cognitive performance in relapsing remitting multiple sclerosis with mild disability. *Neuroimage Clin*. 2020;25:102177. doi:10.1016/j.nicl.2020.102177
216. Tona F, Petsas N, Sbardella E, et al. Multiple Sclerosis: Altered Thalamic Resting-State Functional Connectivity and Its Effect on Cognitive Function. *Radiology*. 2014;271(3):814-821. doi:10.1148/radiol.14131688
217. Schoonheim MM, Meijer KA, Geurts JJG. Network Collapse and Cognitive Impairment in Multiple Sclerosis. *Front Neurol*. 2015;6. doi:10.3389/fneur.2015.00082
218. Roosendaal SD, Schoonheim MM, Hulst HE, et al. Resting state networks change in clinically isolated syndrome. *Brain*. 2010;133(6):1612-1621. doi:10.1093/brain/awq058
219. Basile B, Castelli M, Monteleone F, et al. Functional connectivity changes within specific networks parallel the clinical evolution of multiple sclerosis. *Multiple Sclerosis Journal*. 2014;20(8):1050-1057. doi:10.1177/1352458513515082
220. Cocozza S, Pontillo G, Russo C, et al. Cerebellum and cognition in progressive MS patients: functional changes beyond atrophy? *J Neurol*. 2018;265(10):2260-2266. doi:10.1007/s00415-018-8985-6