

MINI-SYMPOSIUM: White matter damage in dementia

## Cognitive Impairment in Multiple Sclerosis: Clinical, Radiologic and Pathologic Insights

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### Keywords

cognitive impairment, gray matter, imaging, multiple sclerosis, pathology, white matter.

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### Abstract

Cognitive impairment is a common and debilitating feature of multiple sclerosis (MS) that has only recently gained considerable attention. Clinical neuropsychological studies have made apparent the multifaceted nature of cognitive troubles often encountered in MS and continue to broaden our understanding of its complexity. Radiographic studies have started to decipher the neuroanatomic substrate of MS-related cognitive impairment and have shed light onto its pathogenesis. Where radiographic studies have been limited by inadequate resolution or non-specificity, pathological studies have come to the fore. This review aims to provide an overview of the nature of cognitive impairment typically seen in MS and to explore the literature on imaging and pathological studies relevant to its evolution. In particular, the relative contributions of gray (ie, cerebral cortex, hippocampus, thalamus and basal ganglia) and white matter to MS-related cognitive impairment will be discussed and the importance of interconnectivity between structures highlighted. The pressing need for longitudinal studies combining standardized neuropsychometric, paraclinical and radiographic outcomes obtained during life with post-mortem tissue analysis after death is presented.

## INTRODUCTION

Cognitive impairment in MS is complex and multifactorial, with both white (WM) and gray (GM) matter damage implicated in its pathogenesis (54). Neuropsychometric studies have mapped out the landscape of cognitive deficits encountered in the disease, which has helped the interpretation of imaging findings. Magnetic resonance imaging (MRI) studies have been monumental in teasing apart the relationship between the distribution and extent of pathology in the MS brain and how it relates to cognitive impairment. However, inherent limitations of current imaging modalities have made the study of MS post-mortem tissue ever more topical and relevant.

In this review, we will present a broad overview of the nature of MS-related cognitive impairment and highlight some of the neuropsychometric tools used in clinical practice and research settings. We will then explore the contribution of neuroimaging studies in dissecting out the relative impact of GM and WM pathology on MS cognitive impairment by focusing on cognitively relevant neuroanatomic structures. The same approach will be applied to a survey of the neuropathologic literature to aid in the interpretation of rapidly accumulating MRI findings. The emerging role of post-mortem MRI studies in more precisely deciphering pathologic contributions (and their radiographic surrogates) to MS cognitive decline is discussed. The pressing need for longitudinal studies wherein serial neuropsychometric, neuroimaging and paraclinical data is accumulated in life and complemented with post-mortem brain tissue analysis after death is outlined.

## COGNITIVE IMPAIRMENT IN MS

Cognitive impairment is now recognized as a common and substantial consequence of MS. It occurs in 40%–70% of the MS patients, depending on the population studied and the diagnostic methodology and cognitive impairment is known to have a significant impact on quality of life. MS patients who are cognitively impaired are more likely to report loss of self-esteem, participation in fewer social activities and higher rates of divorce (107, 178). Both cross-sectional studies and longitudinal studies indicate cognitive impairment is the strongest predictor of MS patients reducing work responsibilities or leaving the workforce (88, 157, 185).

Classically, the cognitive impairment associated with MS has been labeled as a “subcortical dementia.” Subcortical dementia has been previously described in other neurological conditions such as Huntington’s disease or Parkinson’s disease (205). The clinical presentation differs from cortical dementias such as Alzheimer’s dementia mainly because of the lack of aphasia, apraxia and agnosia (61). In contrast, the subcortical dementias affect information processing speed, memory and executive function and often present as a profound slowing of cognition also referred to as bradyphrenia (61). Instead of cortical lesions or atrophy, the lesions responsible for subcortical dementia are thought to occur in the deep gray matter (basal ganglia and thalamus), brain stem and cerebellum (62). Additionally, psychiatric and personality changes are common with subcortical dementias, specifically cognitive inflexibility (177) and mood disorders such as depression and anxiety, apathy and irritability (1). Yet, as will be discussed

below, the term subcortical dementia implies a disconnect between cortical and subcortical pathologies, which does not truly exist in MS. Further, there is a wide variability in the clinical presentation of cognitive impairment in MS and this presentation does not always fit the concept of subcortical dementia.

Cognitive impairment in MS most frequently presents as impaired information processing speed, as well as impaired immediate and delayed recall or memory (23, 177). Verbal fluency and executive function can also be involved (54). Although disease duration and physical disability do not predict the presence of cognitive impairment, there is a likely a relationship with disease type and time since diagnosis (2, 12). Feuillet *et al* found 80% of clinically isolated syndrome (CIS) patients who met McDonald's (2005) disseminated in space criteria on MRI were impaired on at least one cognitive domain, while 57% were impaired on two or more tests (86, 174). Similar patterns were found in other CIS and recently diagnosed (2–3 years) relapsing remitting (RR) MS patients (98, 180). Achiron *et al* in a large cross-sectional study divided subjects in 5-year cohorts by year of diagnosis and found a decrease in all cognitive domains after the fifth year after diagnosis (2). Cognitive impairment is known to be more frequent and more severe in progressive types of MS than RRMS (2, 3, 177); although studies vary when comparing primary progressive (PPMS) and secondary progressive (SPMS) MS. Comi *et al* reported a higher prevalence of cognitive impairment in SPMS than PPMS (57% vs. 7%); however, this was a small study with only 31 progressive patients included (59). Huijbregts found SPMS were more severely and more frequently impaired than PPMS subjects, who in turn were more impaired than the RRMS group (113). In contrast, larger population-based studies have found 29%–87% of PPMS patients were cognitively impaired (42, 45), and in other studies, the degree of cognitive impairment between SP and PPMS is comparable (74, 91).

In contrast, all studies support the concept that cognitive impairment in MS is progressive with little evidence of improvement once apparent. Duque and colleagues evaluated 44 MS subjects of all types every 3 months over a 2-year period (75). At baseline, 31% were considered impaired, and at 2 years, that increased to 41% with a decrease in verbal memory and processing speed being most prominent. Other short-term (3 years or less) longitudinal studies have found similar results (11, 127). Only one long-term study has been published by Amato *et al* (10). These investigators followed 45 MS subjects over 10 years, who were considered early in the disease as they were on average 1.5 years since diagnosis with Poser criteria. At baseline, 74% were cognitively normal, which decreased to 51% at 4 years and 44% at 10 years. These studies examined predictors of developing or worsening cognitive impairment over time; no characteristic was found to be predictive of worsening cognitive function other than the presence of cognitive impairment at baseline.

Although known to be insidiously progressive in nature, more recently, it has been demonstrated that acute inflammatory lesions, or relapses, can affect cognitive function. Morrow *et al* examined subjects receiving natalizumab monthly who also had the Symbol Digit Modalities Test (SDMT) administered at each infusion visit in the STRATA extension study (158). The authors retrospectively compared 53 subjects with documented relapses to 115 stable controls matched on age, gender and baseline SDMT values at time of study initiation and found SDMT change pre- to post-

relapse was significantly greater in the relapse group than matched stable controls, with a change of 4.0 on the SDMT found to be clinically meaningful. However, this study included all relapses documented in the phase IV study and was not specific to cognitive complaints. A prospective study by the same group examined MS patients with cognitive complaints and compared neuropsychological tests done before, during and after the relapse to matched controls without relapses. Again, a decline on the SDMT was noted during the acute inflammatory phase, on average a worsening of 3.5 points or approximately 6% (25). These studies confirm that acute inflammation also contributes to cognitive function in MS patients.

## TOOLS TO MEASURE COGNITIVE DECLINE IN MS

It is important, when examining cognitive impairment clinically or for research protocols, to ensure the tests being used are appropriate, reliable and valid. In 1991, Rao developed and validated the Brief Repeatable Battery of Neuropsychological tests (BRB-N) for MS, a comprehensive battery that could be administered in 1 h or less (30) (Table 1). The BRB-N was found to have a sensitivity of 71% and specificity of 94% in discriminating cognitively impaired from cognitively intact MS patients (30). Some researchers noted this battery did not have a measure of visual/spatial ability or executive function; often other tests are added to the battery to assess other measures. In 2002, a panel of neuropsychologists and clinical psychologists with expertise in the field of cognitive impairment in MS developed a battery for what they determined to be a minimal neuropsychological examination to assess the principal features noted in cognitive impairment in MS patients (24). This battery, named the Minimal Assessment of Cognitive Function in MS (MACFIMS), was similar to the BRB-N but with additional tests ensuring a more comprehensive assessment but

**Table 1.** Neuropsychometric batteries commonly used in multiple sclerosis. Abbreviations: BVMTR = Brief Visuospatial Memory Test—Revised; COWAT = Controlled Oral Word Association Test; CVLT2 = California Verbal Learning Test—2nd edition; D-KEFS = Delis–Kaplan Executive Function System; JLO = Judgment of Line Orientation test; PASAT = Paced Auditory Serial Addition Test; SDMT = Symbol Digit Modalities Test; SPART = 10/36 Spatial Recall Test (16); SRT = Selective Reminding Test.

Domain	BRN-B	MACFIMS	BiCAMS
Processing speed (auditory) and working memory	PASAT (103)	PASAT	
Processing speed (visual)	SDMT (194)	SDMT	SDMT
Verbal memory (learning and recall)	SRT (41)	CVLT2 (65)	CLVT2*
Visual/spatial memory (learning and recall)	SPART (16)	BVMTR (22)	BVMT*
Verbal fluency	COWAT (36)	COWAT	
Spatial processing		JLO (28)	
Executive function		D-KEFS (66)	

\*For the BiCAMS, only the immediate recall portion of the CVLT2 and BVMTR are included, while both the BRN-B and MACFIMS include both the immediate and delayed recall test.

also increasing the time for administration to 1.5–2 h (Table 1). The panel also recommended, although not included in the battery *per se*, that premorbid cognitive ability, generalized fatigue and mood disorders, specifically depression, should also be measured as these factors can affect performance on objective cognitive measures. Impairment on one test was defined as worse than 1.5 standard deviations below the mean on that test and cognitive impairment was defined as impairment on two domains in the MACFIMS battery. Since that time, multiple studies have supported the lack of effect of generalized fatigue on objective measures of cognitive impairment, although fatigue has a significant impact on the subjective evaluation of cognitive function in MS; thus this symptom should still be considered when evaluating cognitive function. The MACFIMS was subsequently evaluated and found to be a valid and reliable measure of cognitive impairment in the MS population, demonstrating that all 11 neuropsychological outcomes included in the battery significantly discriminated between MS patients and normal controls, as well as between RRMS and SPMS patients (23).

Both of the above batteries are comprehensive, and a recent study comparing the BRB-N to the MACFIMS found both had similar discriminative validity to distinguish MS patients from healthy controls as well as an excellent prediction rate: 79% for the BRB-N and 83% for the MACFIMS (198). However, the time it takes to administer the full battery remains a limiting step. Indeed, there is much ongoing research to develop either a screening test or a screening battery for cognitive impairment in MS. Many studies support the high validity, discriminative ability and test-test reliability of the SDMT, one of the two measures of processing speed included in both comprehensive batteries (23, 73, 159, 175, 198, 217). Yet, there is concern that one test of only one domain will miss those MS patients who are impaired in other domains, specifically memory, which is also frequently affected in MS patients (23, 48, 160). A shorter battery based on the MACFIMS, which can be completed in 15 minutes, has been proposed by Langdon *et al*, the Brief International Cognitive Assessment for Multiple Sclerosis (BiCAMS) battery (131) (Table 1).

## MRI AND COGNITIVE IMPAIRMENT IN MS

MRI is currently the best biomarker available for both diagnosis and monitoring of disease activity in MS patients. Demyelinating plaques, both active and chronic, are correlated with high signal (hyperintensity) on T<sub>2</sub>-weighted sequences on MRI (163, 190). Over time, new T<sub>2</sub> hyperintense lesions may diminish in size as inflammation resolves, yet a permanent T<sub>2</sub> lesion often remains. Overall T<sub>2</sub> volume increases yearly by approximately 5%–10%

(203). On T<sub>1</sub>-weighted images, areas of hyperintensity on T<sub>2</sub> often have corresponding low signal (hypointense) lesions. T<sub>1</sub> hypointensities can resolve, as the T<sub>2</sub> hyperintense lesion resolves. Permanent T<sub>1</sub> hypointense lesions reflect irreversible axonal loss and/or permanent myelin damage (208). Gadolinium, a paramagnetic agent, causes a high signal intensity (hyperintensity) in areas of blood–brain barrier (BBB) breakdown on T<sub>1</sub>-weighted images (104). Gadolinium-enhancing lesions are correlated with pathologic evidence of BBB compromise, allowing infiltration of pro-inflammatory T-cells, and only appear when there is acute inflammation (141). These lesions are transient, lasting approximately 4 weeks, and may precede a new T<sub>2</sub> lesion by hours to days (118, 149). New T<sub>2</sub> lesions and contrast-enhancing lesions correlate with active relapses (67, 111, 118), yet many new lesions appear on MRI that are clinically silent, and are estimated to occur 10× more frequently than clinically relevant relapses (111, 171, 203).

### White matter

#### Inflammatory lesions and cognitive impairment

Hyperintense T<sub>2</sub> lesions have been found to be related to cognitive impairment and correlate better with cognitive impairment better than T<sub>1</sub> or gadolinium-positive lesions (111, 179). Several studies have found a relationship between cognitively impaired MS subject and T<sub>2</sub> lesion volumes (67, 171). Rossi *et al* found the overall anatomical distribution of T<sub>2</sub> lesions in the brain similar between cognitively impaired and cognitively preserved but also found greater WM lesion volume in cognitively impaired vs. cognitively preserved MS patients. Further, the peak of lesion frequency was two times higher in the corpus callosum in the cognitively impaired group. The authors also found the SDMT was significantly correlated with WM lesions and low SDMT scores were associated with high lesion frequency in corpus callosum (183) (Table 2).

### Cortical gray matter

#### Cerebral cortex

#### Cerebral cortical lesions and cognitive impairment

Recently, advances in MRI technology, including new sequences and higher field strength, have led to the identification of cortical lesions in MS. Double Inversion recovery (DIR) sequence utilizes two inversion times to suppress the signal from WM and CSF allowing the images to show superior delineation of cortical GM. Cortical lesions can now be identified and cortical lesion volume has been found to be significantly higher in cognitively impaired

**Table 2.** Radiographic and pathologic featured references by anatomic location.

Region	Subregion	Anatomic structure	Featured radiology references	Featured pathology references
White matter	Subcortical WM	N/A	(67, 111, 171, 179, 183, 187, 202)	(8, 35, 37, 82, 85, 92, 130, 135, 154, 186, 204, 206)
Gray matter	Cortical GM	Cerebral cortex	(9, 15, 43, 44, 50, 136, 155, 164, 191)	(32, 33, 39, 112, 130, 142, 144, 145, 172, 213)
		Hippocampus	(121, 193)	(76, 77, 94, 168)
	Deep GM	Thalamus	(17, 27, 56, 111)	(57, 96, 106, 209, 211)
		Basal ganglia	(17, 29, 38)	(106, 209, 211)

MS patients (44). Calabrese *et al* (2009) reported cortical lesions in both cognitively impaired and cognitively preserved MS patients, similar to the pattern of demyelinating T<sub>2</sub> lesions noted on conventional MRI, but noted that cognitively impaired MS patients had a higher cortical lesion volume and lower cortical volume overall. Regression analysis found both cortical volume and cortical lesion volume to be independent predictors of cognitive impairment (43). Studies using 3T MRI have demonstrated that T<sub>1</sub> hypointense cortical lesions are common in MS patients and predict worse scores on neuropsychological tests of memory, processing speed and verbal fluency (15). Finally, 7T MRI imaging has further demonstrated that cortical lesions are consistently associated with cognitive impairment in MS patients (164). Post-mortem MRI studies have demonstrated that MRI is not sensitive but highly specific in the detection of cortical lesions. Therefore, the true extent of cerebral cortical disease is likely significantly underrepresented using current imaging modalities (191).

### Cerebral cortical atrophy and cognitive impairment

Imaging studies have consistently found that measures of brain atrophy on MRI correlate better with clinical disability than the number of volume of T<sub>2</sub> lesions (87, 115). Studies have used brain parenchymal fraction (BPF), a measure of the ratio of brain parenchymal tissue volume within the total brain surface volume on MRI, to quantify the amount of atrophy, which has been found to be accurate and reliable with good reproducibility (87, 110, 184). Kassubek *et al* compared BPF measures of MS patients with healthy controls, controlling for age and gender (116). MS patients had significantly lower BPF measures than controls; BPF was also significantly correlated with disease duration ( $r = -0.6$ ) and EDSS ( $r = -0.5$ ), but not with relapse rate or lesion load measures (50). This data supports BPF, or atrophy, as a measure of degeneration or permanent damage. Although atrophy of both WM and GM are noted over time, MRI studies support GM atrophy as the driving force behind whole brain atrophy. Cross-sectional studies comparing early MS patients to healthy controls confirm a significant brain volume reduction compared with healthy controls, but also find a greater reduction in GM than WM when controlling for age and gender, as well as a lack of correlation between brain atrophy measures and T<sub>2</sub> lesion load (50, 202) (Figure 1). Early MS patients followed for 4 years were found to have GM atrophy occurring at a 3.4× greater rate in the group of first demyelinating event patients who converted to MS vs. those who did not. GM atrophy also occurred 8.1× greater in RRMS, 12.4× greater in RRMS converting to SPMS and 14× greater in SPMS patients. In contrast, WM atrophy occurred at a similar rate in all MS types (3× greater than normal controls) (27).

A similar link has been described between atrophy measures and cognitive function. Whole brain atrophy has also been strongly linked with cognitive impairment in the MS population. MS patients who are cognitively impaired are found to have smaller overall brain volumes as well as GM (136) and WM volume (187). Cross-sectional studies in MS patients have found performance on a test of processing speed to be correlated with BPF (27, 109, 188). Additionally, longitudinal studies also found that MS patients with greater brain atrophy worsened on cognitive tests over time more than MS patients with stable or improved cognitive function (155). Further studies have shown it is cortical volume that is most

important in cognitive impairment. Amato *et al* examined 41 MS patients within 10 years of diagnosis and found MS patients had decreased neocortical volume compared with normal controls and MS patients with cognitive impairment had decreased neocortical volume compared with MS patients without cognitive impairment (9) (Table 2).

### Hippocampus

There are also studies supporting the role of hippocampal atrophy in cognitive impairment. Sicotte *et al* examined the absolute volume of both hippocampi and its segments in early RRMS, SPMS and healthy controls compared with performance on memory tasks. Both types of MS had lower total hippocampal volume when compared with healthy controls, which was localized to the CA1 regions in RRMS and more extensively involved in SPMS. An inverse relationship was found between the number of trials needed to learn a new list of words and total hippocampal, CA1 and subiculum volume (193). This pattern of selectivity vulnerability in CA1 is reminiscent of that seen in anoxia, epilepsy and dementia (reviewed in (14)). Koenig *et al* reported a 6%–7% smaller hippocampal volume in MS patients compared with normal controls, and a smaller hippocampal volume in MS patients who were impaired on measures of memory and processing speed (121) (Table 2).

### Deep gray matter

The possibility that deep GM structures, as opposed to cortical GM, play a role in cognitive dysfunction has also been of interest. Deep GM matter consists of the thalamus, hippocampus, nucleus accumbens and basal ganglia, which encompass the caudate, putamen, globus pallidus and amygdala (see relevant pathology sections for further anatomic details).

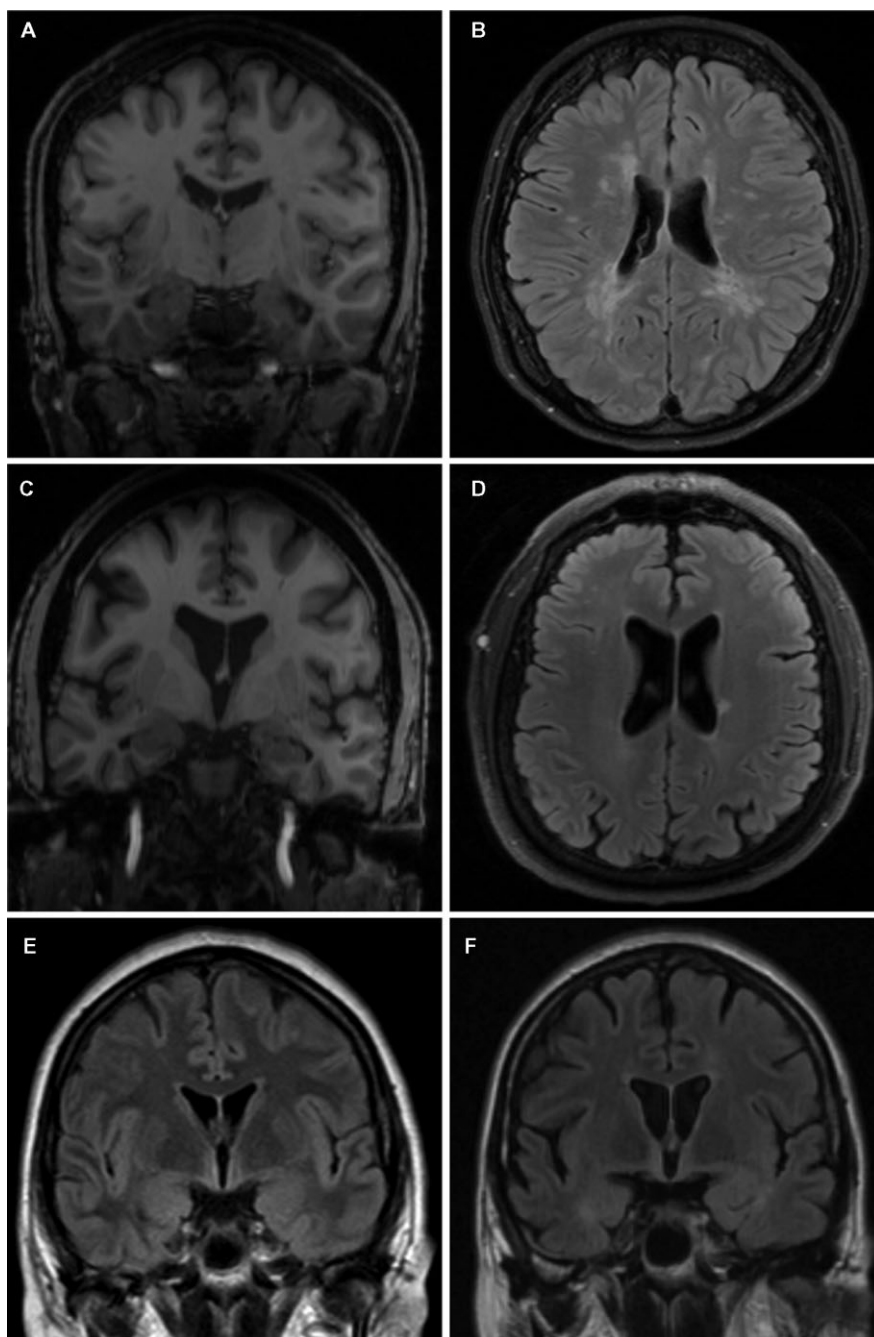
### Thalamus

Some studies have used third ventricular width (TVW) as a measure of whole brain atrophy. Larger ventricles, as measured by ventricular width, indicate a larger amount of cerebrospinal fluid (CSF) and less brain tissue. However, TVW could alternatively represent selective atrophy of the thalami, which border the third ventricle. When comparing MS patients with healthy controls, TVW was a strong predictor of impairment on tests of memory and processing speed (27). Additionally, central atrophy, measured as the percent of central CSF to total intracranial volume, had a strong correlation to cognitive performance (56). If the volume of the thalami is examined directly, it is lower in MS patients compared with normal controls and associated with impairment on tests of processing speed, working memory and visuo-spatial memory (111). Finally, the thalamus was significantly smaller in MS patients with impaired processing speed, verbal fluency, verbal and visuo-spatial learning and executive function (17) (Table 2).

### Basal ganglia

Basal ganglia T<sub>1</sub> hypointensity and T<sub>2</sub> lesion volume were both found to be strong predictors of cognitive impairment in MS patients (38). Similarly, the bicaudate ratio, the ratio of the width





**Figure 1.** Cortical atrophy, lesion burden and cognitive impairment in MS. Coronal (A, C, E, F) and axial (B, D) views of MRI brain scans from RRMS patients without cognitive impairment (patient 1—A, B; patient 2—C, D) and with cognitive decline (patient 3—E, F). Patient 1 demonstrates relatively preserved brain parenchymal volume (A) despite moderately significant WM lesion burden (B). Conversely, patient 2 has evidence of significant globalized cerebral atrophy (C) in the absence of a significant WM lesion load (D). Patient 3 shows radiographic evidence of progressively worsening global cerebral atrophy over 18 months despite disease-modifying therapy and relative stability of WM lesion burden. These images highlight the complex relationships between GM and WM pathology and cognitive impairment in MS. A, C, T1-weighted sequences; B, D, E, F, T2-FLAIR sequence.

of the ventricles between the two caudate to the overall brain width, was significantly greater in MS patients than healthy controls and was a strong predictor of impairment on tests of processing speed (29). Finally, impairment on tests of verbal fluency and verbal learning/memory was also significantly associated with lower volume in both caudate nuclei (17) (Table 2).

## COGNITIVE RESERVE

Cognitive reserve is defined as “the hypothesized capacity of the mature adult brain to sustain the effects of disease or injury

without clinical symptoms, but sufficient to cause clinical symptoms in an individual possessing less cognitive reserve” (197). It is the ability of the brain to tolerate pathology related to an underlying disease process without exhibiting overt signs or symptoms, allowing those with greater baseline cognitive ability to adapt or compensate for changes caused by either normal aging or damage caused by an underlying disease (117, 214). Thus, those with greater cognitive reserve would require more extensive pathology to cause the same degree of impairment, leading to the ability to cope with pathology better than those with less reserve. As mentioned previously, the onset of impairment does not correlate well

with disease duration, can be present at initial diagnosis and does not necessarily follow the same pattern of severity as physical disability (12, 86, 98). The reason for this variability is unknown but may be partly explained by cognitive reserve. A case control study of 43 MS and normal controls matched on level of education, found that the low education MS group performed worse than controls on almost all tests, while the higher education MS group differed from controls only on measures of processing speed and attention. Additionally, the cognitive performance of MS patients was correlated with years of education while the control groups' performance did not differ based on education level (34). In a study of cognitive performance and third ventricular width (TVW) as a measure of brain atrophy, Benedict *et al* found that education level was correlated with each measure of atrophy (27). Sumowski and colleagues (199) investigated whether the adverse impact of brain atrophy on information processing speed and efficiency would be moderated by cognitive reserve. In 38 patients, cognitive function was assessed using the SDMT and Paced Auditory Serial Addition Test (PASAT), measures of processing speed, and measured brain atrophy using TVW. Regression models showed that the expected relationship between brain atrophy and cognitive processing was moderated by estimated pre-morbid intelligence. The study also showed a significant interaction between atrophy and cognitive reserve such that the impact of atrophy on cognition was attenuated at higher levels of cognitive reserve. In other words, among patients with more severe brain atrophy, those with greater cognitive reserve had a better performance on formal cognitive testing. The same was not true of patients with minimal atrophy. Additionally, a longitudinal study examining 90 MS patients over a mean of 5 years, using education as an indicator of cognitive reserve, found those MS patients with more than 14 years of education ("high reserve") showed no significant change on the SDMT over time, while those with low levels of education (<9 years, "low reserve") declined significantly over time (26).

## **PATHOLOGY AND COGNITIVE IMPAIRMENT IN MS**

Pathology studies have played an important role in providing insight into the possible neuroanatomic substrate of cognitive impairment in MS and helping with the interpretation of MRI findings. From a pathologic point of view, MS lesions are heterogeneous in nature even within a given anatomic location and their distribution and size are highly variable between patients. Each MS lesion may have an impact on cognition through various mechanisms including chronic inflammation and/or demyelination, disruption of the neuronal/axonal compartments, oxidative stress, alterations in the BBB and cerebral metabolism and blood flow, and resultant changes in cortico-cortical and cortico-subcortical connectivity to name a few (114). That being said, the links between MS pathology and cognition remain poorly understood. This is largely because of frequent reports of neuroimaging abnormalities to which cognitive impairment has been attributed (eg, cortical, hippocampal, thalamic atrophy and subcortical WM pathology), also being found in "cognitively normal" MS sufferers. Further, the exploration of clinicopathological correlations in MS-related cognitive impairment has been hampered by the application of inconsistent neuropsychometric protocol(s), variable definitions of cognitive impairment and varying MRI measures, as previously highlighted.

The cross-sectional nature of MRI studies and the lack of specificity of findings on the impact of surrogate markers of MS pathology are problematic. The impact of disease-modifying therapies on the evolution of cognitive decline is not accounted for adequately adding yet another layer of complexity.

Pathology studies have filled some of these important gaps, though much work is needed. The confounding issues of lesion distribution/extent, cross-sectional ascertainment and poor clinicopathologic correlations limit the ability of pathology studies to explain MS-related cognitive impairment (Figure 2, Table 2).

In the next section, we will highlight the neuropathology of key anatomic structures implicated in MS-related cognitive impairment.

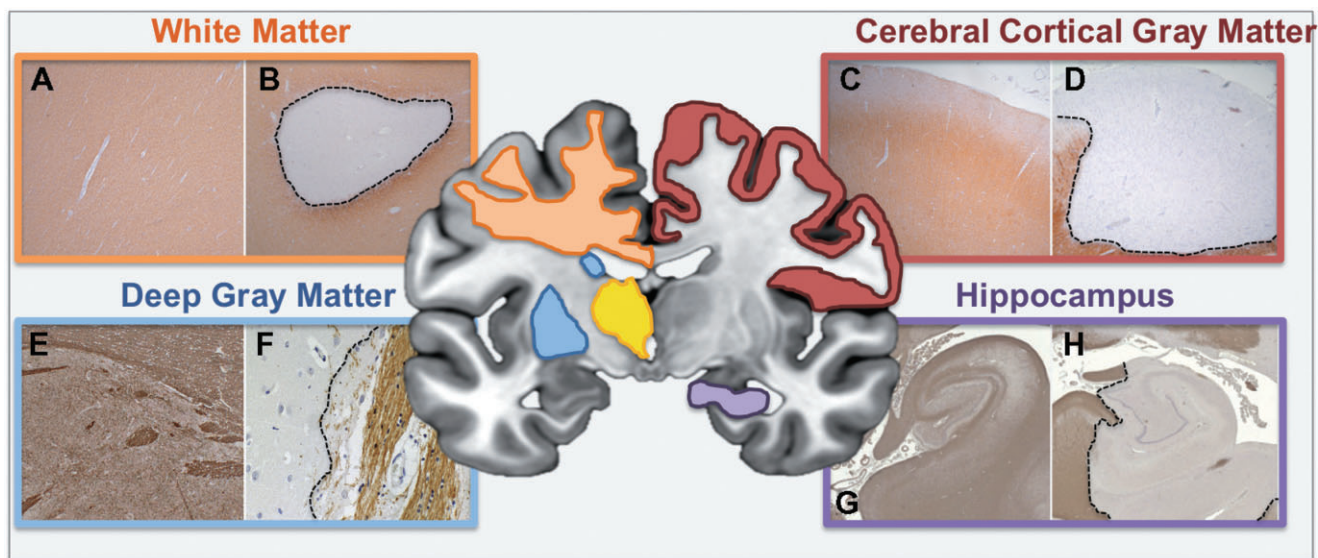
### **White matter pathology and cognitive impairment in MS**

WM consists of glial cells that support aggregations of axons linked by common cortical and/or subcortical origins/destinations grouped into association, striatal, commissural and projection fiber bundles. We will next review the pathology of WM and its involvement in cognitive dysfunction in MS.

### **White matter demyelination and inflammation**

There is a spectrum of WM abnormalities recognized in MS, ranging from lesional WM (acute and chronic), diffusely abnormal (or dirty appearing) WM (DAWM) and normal appearing WM (NAWM). WM lesions have long been considered the pathologic hallmark of MS and can be easily detected both *in vivo* by MRI and upon post-mortem examination (132) (Figure 2). Acute inflammatory demyelination is widely accepted as the pathologic surrogate for clinical relapses, which demonstrate radiographic evidence of gadolinium enhancement indicating disruption of the BBB as outlined in the MRI section of this review. From a histologic perspective, these focal lesions are characterized by recruitment of monocytes and lymphocytes, which traverse the disrupted BBB into the perivascular space where ongoing immunological interactions take place (102). Early infiltration of macrophages/microglia engulf myelin sheaths with or without the deposition of complement or immunoglobulins (4, 40, 186). The distribution and extent of the microglial inflammatory reaction defines the "stage" of the lesion; active lesions are rich in macrophages at their lesional core, chronic (or border) active lesions show abundant macrophages at their margins, and chronic inactive lesions have minimal, if any, macrophage infiltration (72, 95).

CD8+T-cells typically outnumber CD4+ T-cells within the MS lesional milieu, with the latter being the predominant cell type in the perivascular space (35). Pro-inflammatory IL-17 expressing CD8+ and CD4+ cells (Th-17 cells) are thought to play a pathogenic role given their abundance in acute lesions and relative paucity in more chronic lesions (206). B-lymphocytes and/or plasma cells are also present, and become particularly prevalent in progressive disease stages (92, 144). Recent work has elegantly demonstrated the co-localization of complement and immunoglobulin on oligodendrocytes and axons suggesting that complement-mediated damage may be inciting factors in inflammatory demyelination, oligodendrocyte loss, axonal injury and damage, and reactive astrogliosis (186). Remyelination of WM lesions is highly variable



**Figure 2.** White and gray matter pathology in MS. Schematic diagram outlining the anatomical regions of WM and GM pathology covered in this review (white matter—orange box; deep gray matter—blue box; cerebral cortical GM—red box; hippocampus—purple box). Myelin immunolabeled sections illustrate normal or normal appearing WM/GM (left panel within each colored box—A, C, E, G) or demyelination (right panel in each colored box—B, D, F, H). Specifically, well-demarcated

subcortical WM (B), cerebral cortical subpial (D), caudate GM (F) and hippocampal mixed GM/WM (H) lesions are presented alongside MS subcortical NAWM (A), MS cerebral cortical NAGM (C), non-neurologic control caudate nucleus (E) and non-neurologic control hippocampus (H), respectively. A–D. Proteolipid protein immunolabeling. E–H. Myelin basic protein immunolabeling. E, F. Adapted from (208). G, H. Adapted from (166) and used with permission.

between patients and within lesions of the same patient (170) and is inefficient compared with that, observed in cerebral cortical GM (122, 156). Myelin deposited from remyelination is susceptible to “second-attack” demyelination, especially around lesional borders (37). The resulting disruption of WM fiber bundles has potential to have a deleterious impact on cognition.

While WM lesions can occur anywhere throughout the neuraxis, their distribution is certainly not random with periventricular, centrum semiovale and corpus callosal WM involvement being common (64, 89). Subcortical lesions stemming from small vessel disease can mimic the distribution of those seen in MS, and in fact, can share histopathologic features reminiscent of MS plaques. Some of these include alterations in BBB permeability, increased microglial activity, oligodendrocyte drop out and astroglial activation (reviewed by Wharton *et al* in this issue). The established cognitive manifestations of small vessel disease, including reduced information processing speed, and difficulties with attention and concentration, make it plausible that WM involvement in MS may have a similar clinical effect given the nature and overlapping distribution of lesions from both conditions (81).

Outside of lesions, myelin in MS WM typically displays a spectrum of pathologic change ranging from NAWM to DAWM. NAWM is different from WM in control tissue given evidence of diffuse parenchymal and perivascular T-cell inflammation (6, 7), microglial activation with upregulation of major histocompatibility complex expression and gliosis (8, 99, 130). Abnormalities in endothelial tight junctions (120, 173), extracellular matrix proteins (196) and enhanced perivascular expression of matrix metalloproteinases (138), have been described, as has BBB permeability (120). Disruption of the BBB in NAWM allows leakage

of large molecular weight proteins, such as fibrinogen, into the WM compartment triggering a deleterious inflammatory cascade (137). Davalos and colleagues recently demonstrated that BBB-mediated perivascular fibrinogen deposition instigates a rapid and sustained microglial response resulting in release of reactive oxygen species that damages axons, even in the absence of overt demyelination (63). While pathology studies have not been in a position to compare specific pathologic changes to cognitive phenotypes, NAWM abnormalities as defined on MRI (as opposed to lesion burden) correlate with atrophy, disability and impaired cognition (reviewed in (153)).

Diffusely abnormal WM (DAWM), otherwise known as “dirty appearing” WM, refers to WM myelin with hyperintensity on proton density and T<sub>2</sub>-weighted imaging intermediate between that of NAWM and focal WM lesions (154). Typical locations of DAWM are adjacent to periventricular plaques and occipital WM, although they can affect any part of WM including areas distant to lesions (154). Pathologically, DAWM shows selective reduction of myelin phospholipids, as detected by Luxol Fast Blue and Weil myelin stains, with relative preservation of myelin proteins (154). Axonal loss is also present but does not feature to the same extent as the myelin phospholipid abnormalities, which are defining (134, 153). The relationships between DAWM and lesional WM, NAWM and neurodegeneration remain up for experimental discovery. Whether WM signal change (leukoaraiosis) (167) noted in ageing, subcortical vascular cognitive impairment (189) and Alzheimer’s disease (AD) (101) share pathogenic mechanisms with DAWM evolution in MS warrants evaluation. If so, this could have important implications in the understanding of MS-related cognitive impairment.



### White matter axonal loss

Axonal injury and loss are established features of MS WM pathology and likely contribute significantly to the irreversible cognitive decline often seen in the disease (31, 201). Ferguson *et al* first described axonal injury in MS WM lesions via  $\beta$ -APP immunohistochemistry, which refocused the field onto the neurodegenerative aspects of the disease (85). Several studies have replicated these findings and have asserted the relationship between acute axonal injury (and loss) and inflammation both within and outside areas of demyelination (13, 68, 140, 204). Axonal loss can be both an early and late feature, and its extent can reach 40%–80% (31, 69, 82). Small diameter axons are preferentially affected, with WM tracts relevant to cognition, such as the corpus callosum, commonly involved (69, 82, 130, 200).

The cause of axonal injury and loss is probably multifactorial. Axons, stripped of myelin and surrounding glial trophic support, are susceptible to degeneration especially when electrically active (161, 195, 216). CD4+ and CD8+ T-cells in active WM lesions have been shown to be neurotoxic *in vitro* via direct cell contact-dependent mechanisms (97). It is established that activated macrophages and microglia contribute to axonal damage in lesions (85) but the diversity of phenotypes of these cells (151) point to their involvement in neurotoxicity and neuroprotection (53). The observation that microglia can strip cortical neurons of inhibition synapses leading to neuronal survival is a recent example of the latter (52). The fact that peripherally derived macrophages compared with resident microglia can perform differing roles in driving lesion pathology and axonal loss further complicates matters (139). There are also longer standing consequences of inflammatory demyelination on the axonal unit, including redistribution of ion channels after demyelination (60), upregulation of the acid-sensing ion channel-1 with subsequent aberration of sodium and calcium regulation (212), chronic mitochondrial injury with resultant oxidative stress and energy failure, and dysregulation of iron homeostasis (106), among others. The predisposition of remyelinated axons to be the target of “second-hit” demyelination adds yet another layer of complexity to the pathogenesis of lesion-associated axonal injury and loss (37).

The presence of axonal injury and loss in both NAWM and DAWM broadens the traditional plaque-centric view of its pathogenesis (69, 82, 135). Where axonal loss does occur in NAWM and DAWM, it can be partly explained by secondary (Wallerian) degeneration stemming from injury from acute lesions in structures such as the corpus callosum (83). However, the lack of significant correlations between demyelination and axonal loss in other WM tracts draws attention to the possibilities that inflammation, diffusible cytotoxic factors (144, 145), glutamate excitotoxicity (126) and/or axon-targeted immune-mediated injury (147) rather than myelin loss per se, may be the culprit process(es) (13, 68, 112). One must also consider that axonal vulnerability to inflammatory demyelination may intrinsically differ between different WM tracts within the same patient (70). A better understanding of the contribution of these pathological processes to axonal demise in WM tracts important for cognition is urgently needed.

### Cortical gray matter pathology and cognitive impairment in MS

Post-mortem analysis of the MS brain has established that GM structures throughout the central nervous system, such as cerebral cortex, thalamus, hippocampus and basal ganglia, are commonly affected and have important consequences on cognitive function. The following sections will review the evidence for involvement of each of these GM structures in MS pathology and explore their potential impact on neuropsychometric outcomes.

#### Cerebral cortex

The cerebral cortex is an organized, laminated structure consisting of six layers defined by their predominant cell type. The cortex contains two types of neurons: principal (mostly pyramidal) neurons (80% of total population) and local interneurons (20% of the total population) that are surrounded by a complex network of glial cells and embedded in an intricate pattern of myelin and extracellular matrix proteins. Disruption of neuronal connections, glial support networks and myelination pattern are the substrate for several neuropsychiatric conditions, of which many have cognitive dysfunction as a core feature (21). It is feasible that similar processes are involved in MS-related cognitive impairment.

**Cerebral cortical demyelination** Involvement of cortical demyelination in MS has been a long recognized feature that has only gained particular attention over the last two decades. Descriptions of cerebral cortical demyelination were furnished in the late 19th and early 20th centuries by several eminent neuropathologists, including Dawson among others (64). In the early 1960s, Brownell and Hughes provided an authoritative addition to the literature through the examination of 22 post-mortem MS cases in which they macroscopically evaluated the extent and distribution of cerebral lesions (39). Of the 1594 lesions identified, 80 (5%) involved the cortex and 265 (17%) were situated at the junction between cortex and WM. The authors astutely recognized that the extent of cortical GM demyelination was likely significantly under-detected in their hands (39), a limitation that standard histochemical techniques and conventional *in vivo* imaging modalities would only partially address over subsequent decades. It was only since the relatively recent application of immunohistochemistry for myelin proteins in MS cortical tissue that the severe extent of cortical demyelination was appreciated. This, in combination with an increased awareness of cognitive dysfunction in MS patients and its relationship to GM structural change detected on imaging, dampened the WM-centric view of MS pathology and brought to the fore the role of (cortical) GM pathology in the pathogenesis and phenotypic expression of the disease.

Cortical demyelination can occur at an early disease stage (142) and has been documented in every clinical phenotype, being most prevalent and extensive with advanced disease duration and in progressive disease (128, 130). Cortical regions that appear to be particularly prone to demyelination include the cingulate gyrus, temporal and frontal cortices, and the depths of sulci. These areas lie in regions of relative CSF stasis leading to the hypothesis that neurotoxic mediators in the CSF influence cortical demyelination (112). In contrast, the paracentral lobule, occipital lobe and



primary motor cortex display comparatively smaller amounts of pathology (129). That said, no cortical region is universally spared from the MS disease process and indeed in progressive MS the majority of the cortical ribbon can be demyelinated (96, 128) although this can vary markedly between patients.

Several classification systems to describe the topography of cortical demyelination have been proposed (32, 119, 172). Four major lesion types in the MS cortex have been defined: type I (leukocortical) lesions span deeper GM layers with involvement of subcortical WM at the gray/white junction; type II (intracortical) lesions are limited to the cortex (sparing subpial and subjacent WM boundaries) and are typically small and centered around small veins; type III (subpial) lesions extend from the pial surface into superficial cortical layers; and type IV lesions originate from the pial surface and span the entire cortical width without involvement of underlying WM although extension into subcortical WM has been described (142). These lesion types not only differ in location but also in their extent of cortical area coverage with subpial lesions (types III and IV) being most extensive and intracortical lesions, although most numerous, being smallest. Both the location (cortical layer(s) and hemispheric position) and extent of cortical demyelination are likely to play critical roles in the evolution of cognitive impairment (51) (Figure 2).

The distribution and extent of cortical demyelination can vary markedly between MS patients, the reasons for which are unclear (129). It has recently been demonstrated that heterogeneity in cortical pathology in MS can be explained partly by genetic variation at the *HLA-DRB1\*15* locus, with younger individuals positive for this allele having significantly more severe demyelination than those who are negative (215). The observation that cortical MS disease may be more severe in a discrete genotype group is supported by evidence of significantly greater cerebral hemispheric T<sub>2</sub> lesion burden, decreased levels of N-acetyl aspartate (a neuronal marker) and more severe cognitive impairment in MS patients carrying the *HLA-DRB1\*15* allele (166). Given the limited resolution to detect cortical lesions, further imaging and pathological studies exploring the role of genetic factors on cortical lesion burden and topography and their relationship to cognitive dysfunction are warranted.

**Cerebral cortical remyelination** Cortical demyelination is not a permanent outcome in MS. There is increasing evidence to suggest that cortical remyelination is frequent and can be extensive. In a comprehensive study by Albert *et al* using light and electron microscopy, cortical remyelination was detected in 28 of 29 (95%) cases and was more extensive compared with WM lesions from the same patients (5) but the investigators did not control for lesional age. To address this confound, Chang *et al* examined leukocortical lesions (where GM and WM involvement are presumably temporally congruent) and found that GM had significantly greater oligodendrocyte generation and remyelination compared with subjacent WM (49). Differences in the extent of astrogliosis and hyaluronin deposition, factors known to frustrate oligodendrocyte differentiation and myelination, were thought to account for these findings (49). An intriguing alternative possibility is that there may be factors in the cortex favoring oligodendrocyte reactions. In support of this view, some have argued that the commonly observed sparing of subcortical myelin

in leukodystrophies may be secondary to repeatedly regenerated myelin of subcortical U-fibers because of proximity to efficient myelin-generating cortical oligodendrocytes rather than because of myelin sparing (156). Whether subpial (type III) demyelinating lesions demonstrate similar remyelination potential compared with the other lesional types remains unanswered and will only be elucidated when an accepted staging method for cortical lesions is devised (49).

The relationship between the extent of cerebral cortical remyelination and age remains contentious. Recent work by Yates *et al* and others have demonstrated that MS cases with longer disease duration and later age at death have more abundant cortical myelination (169, 170, 215). Given that actively remyelinating oligodendrocytes are commonly encountered in patients over 70 years of age with long-standing disease duration (122), it is likely that remyelination extends into old age and may contribute to “cognitive reserve.” While experimental data suggests that oligodendrocyte remyelinating potential decreases with age (100), this phenomenon is undoubtedly influenced by the changing extent and quality of inflammatory activity associated with aging (165). This may subsequently impact the susceptibility of newly formed myelin from “second-hit” demyelinating attack in older patients (37). The observed substantial interindividual variation in remyelinating capacity irrespective of age implicates underlying genetic and/or environmental factors (170, 182).

**Cerebral cortical inflammation** Pathological studies evaluating inflammation in MS have traditionally focused on WM regions throughout the neuraxis (181). This WM exploratory bias has been fueled, in part, by the notion that GM structures, such as cerebral cortex, have a paucity of inflammation even in active disease stages. Recent data have challenged this view highlighting how inflammation within the cerebral cortex and overlying meninges can be severe and extensive, and contribute to other cortical pathological features likely important for cognitive function.

Cortical parenchymal inflammation within lesions and NAGM is variable but routinely less severe than underlying WM. Leukocortical lesions contain more pronounced inflammatory infiltrates compared with their intracortical and subpial counterparts (32, 33, 112, 123, 130, 142, 172, 211). In chronic MS, cortical lesions residing solely within the GM compartment show infrequent T-cell lymphocytes, which when present, are typically restricted to the perivascular space. B-cell infiltrates are rare. Varying degrees of activated microglia are also present, which typically take on a ramified appearance. Myelin-laden macrophages are atypical. There is a widely held view that they are not associated with BBB breakdown or complement deposition (32, 172, 207). The ascertainment bias of post-mortem tissue derived from end-stage progressive MS cases demands consideration in the interpretation of these findings.

On the flip side of the ascertainment spectrum, a recent biopsy study has broadened the view of cortical demyelination in early MS (142). Cerebral cortex, sampled “en passant” of the stereotactic retrieval of WM lesions suspicious for tumor, revealed demyelination associated with pronounced CD3+ T-cell infiltration and macrophages with myelin-inclusions in the setting of radiographic evidence of BBB disruption. The presence of complement deposition was not studied. While the majority of patients studied

later fulfilled criteria for a diagnosis of MS, there should be cautious extrapolation of these findings to understand the pathogenesis of the cortical MS lesion. The clinical and radiographic presentations justifying the need of biopsy suggest atypical MS presentations, and therefore, possibly less typical pathology. Further, the extent to which the cortical findings were influenced by the juxtaposed fulminant WM pathology that prompted tissue biopsy in the first place is not certain.

The contrasting features of cortical pathology in early and late disease stages leave unanswered important questions about the pathogenesis of the cortical lesion. Involvement of the BBB in MS cortical lesions is contentious despite the majority of these being centered on veins (152). Some investigators propose that CNS inflammation becomes compartmentalized behind a “closed” BBB once the disease transitions from a phenotype of focal inflammatory demyelination to that of more diffuse cortical disease as encountered in progressive disease (128, 130). The detection of marked endothelial tight junction abnormalities in cortical NAGM derived from SPMS and PPMS cases (137) and in cortical lesions despite minimal inflammation in a chronic murine model of the disease challenge this conceptual framework (80).

Microglial responses may prove a key culprit, independent of BBB disruption. Microglial activation can be severe and extensive in MS cortical lesions, with a recent report suggesting that its severity is influenced by *HLA-DRB1\*15* status even in long-standing cases (125, 215). The functional consequences of increased microglial burden are not certain however. Microglia respond to cytotoxic factors from the subarachnoid space (implicating neurovascular communication) (102) and show age-related dysregulation (71), both of which may be relevant when weighing the role of these resident macrophages on cortical pathology and age-related cognitive decline. Given that chronic disruption of the neurovascular unit and microglial activation contribute to both GM and WM pathology in diseases wherein progressive cognitive decline is a hallmark, such as AD (reviewed in (79)), the possibility of similar parallel processes being operative in MS deserves attention. Complicating matters, recent work has demonstrated that activation of microglia in the cortex may confer neuronal protection via stripping of inhibitory synapses (see cerebral cortical neuronal and synaptic loss section) (52).

While features of cortical pathology appear to vary between early and late MS, meningeal inflammation is a unifying feature (68, 142, 145). Numerous studies have reported substantial meningeal immune cell infiltrates composed of T- and B-cells and macrophages, and noted their relationship to underlying cortical GM pathology both in progressive MS (130, 144, 192) and early-stage cortical biopsies (142), although these relationships have not been universally found (5, 123, 215). Organization of meningeal B-cell inflammation in what resemble tertiary lymphoid-like structures is a replicated feature (112, 144, 192). The extent of meningeal inflammation has been linked to underlying cortical demyelination, inflammation and a gradient of neuronal loss that is greatest in superficial cortical layers closest to meninges in both demyelinated and normal appearing GM (see section below) (145). These results point toward a cytotoxic milieu in the meningeal/CSF compartment, an idea supported by detection of enhanced meningeal gene expression of tumor necrosis factor and interferon-gamma, cytokines known to induce subpial cortical pathology similar to that seen in MS (93).

**Cerebral cortical neuronal and synaptic loss** Neuronal loss is a notable feature of the landscape of MS cortical pathology. In a seminal report, apoptotic neurons and transected neurites (ie, dendrites and axons) were observed in cortex, with the former concentrated in large, pyramidal neurons in cortical layers 3 and 5 and the extent of the latter associated with lesional inflammatory activity (172). Subsequent studies have built upon these findings demonstrating significant neuronal loss (approximately 10%–30%) occurs within lesions and in normal appearing GM (145, 210, 213) with specific neuronal subpopulations (ie, interneurons expressing parvalbumin in cortical layer 2) being particularly vulnerable (58). A gradient of neuronal loss has been observed, with most severe loss in superficial cortical layers especially in MS cases where overlying meningeal inflammatory lymphoid-like follicles are present (145). In early biopsy-derived MS tissue where pronounced cortical inflammatory demyelination features, neuronal and axonal injury is seen (142). Synaptic loss is another element of cortical pathology, with one study showing a 47% reduction in synaptophysin in lesional cortex (213).

Pathology of the neuronal-axonal compartment is not restricted to cortical lesions. Several studies have now convincingly shown that neuronal mitochondrial dysfunction and cellular drop out is also detected in NAGM distant from lesions suggesting that myelin loss per se is not the driving substrate (47, 78, 145). Rather, mounting evidence suggests that parenchymal inflammatory cellular reactions and/or meningeal-derived diffusible soluble cytotoxic factors play an important role in the disruption of the neuronal-axonal unit.

The chronic inflammatory milieu in MS likely drives the observed deficiencies in oxidative phosphorylation and GABAergic neurotransmission. Gene and protein expression studies of normal appearing cortex have demonstrated defects in oxidative phosphorylation and functional reduction in mitochondrial respiratory chain complex I and III (78). In combination with the observation of significantly increased levels of mitochondrial DNA deletions in cortical neurons (46), the changes in oxidative phosphorylation are thought to set the stage for neuronal-axonal energy failure leading to a neurodegenerative fate. In contrast, decreased inhibitory GABA transmission may confer neuroprotection through the upregulation of neuroprotective pathways (53). Studies have demonstrated that neuronal survival is enhanced by synaptic firing of NMDA receptors, a process facilitated by the inhibition or temporary disruption of GABA-ergic inhibition (53). Recent elegant experiments by Trapp and colleagues have built upon this work establishing that activated microglia can migrate to and displace inhibitory GABA-ergic synapses from cortical neurons. The resultant increased synchronized cortical neuronal firing increased expression of anti-apoptotic and neurotrophic molecules and decreased cortical neuronal apoptosis following injury (52). These findings may be particularly relevant to cortical neurons in superficial layers I–III, which are involved in cortico-cortical connections, given their vulnerability to subpial demyelination and meningeal-derived cytotoxic soluble mediators as outlined above.

**Cognitive implications of cerebral cortical pathology in MS** Direct clinicopathological evidence linking features of cortical pathology to MS-related cognitive decline are lacking given insufficient clinical information and/or cognitive testing late in life

typical of autopsy series. That being said, features of MS cortical pathology when married with what is known about cortical function provide insight into MS-relevant cognitive phenotypes. The preferential affectation of prefrontal, middle temporal and cingulate cortices to demyelination likely contribute to deficiencies in executive function, learning, memory retrieval, and emotion formation and processing often encountered in the disease. The prevalence of demyelination, cytotoxic inflammation and neuronal loss in both superficial (I–III) and deep (V–VI) cortical layers is of particular relevance to cognitive disturbance given these layers are important for cortico-thalamo-cortical activity, cortical binding, synchronization and plasticity, which impact cognitive function. Chronic microglial activation, while likely a driver of free radical generation and excitotoxic neuronal injury, may confer cognitive protection by its anti-apoptotic influence on neurons and promotion of cortical synchronization via stripping of GABAergic inhibitory synapses. The positive influence of microglia on amyloid deposition in the MS brain may additionally protect from Alzheimer's-related cognitive decline.

Taken together, the complex interplay between the distribution, nature and extent of demyelination, inflammation, neuronal-axonal injury and loss in the MS cerebral cortex, contribute to MS-related cognitive decline and differentiate it from other degenerative dementia syndromes. While cerebral cortical atrophy is a recognized radiographic surrogate of cognitive decline in MS, so too is hippocampal atrophy (see MRI section above). The pathology of this archaocortical structure will be reviewed next.

## Hippocampus

The hippocampal formation is located in the medial temporal lobe and includes the dentate gyrus, the hippocampus proper (including CA1, CA2, CA3 and CA4 subfields) and the subiculum. These structures form part of a predominantly unidirectional glutamatergic excitatory pathway that is complemented by parallel and reciprocal connections within the hippocampus that form a functionally organized circuit (20). The hippocampal formation forms a circuit with neocortical structures subserving diverse function with memory formation, maintenance and retrieval being key elements. Emotional memory processing (via amygdala, orbitofrontal, medial prefrontal cortex) and visuospatial memory and navigation (via precuneus and posterior cingulate cortex) are additional features (20).

**Hippocampal demyelination** Aside from incidental descriptions of hippocampal demyelination in a few atypical presentations of MS (90, 162), the first systematic assessment of hippocampal demyelination was carried out in 2007 (94). In this study, a total of 37 lesions were found in or around the hippocampus in 15 of 19 (78.9%) MS cases sampled. Both intrahippocampal lesions (involving dentate gyrus and/or ammon horns) and mixed GM and perihippocampal WM lesions featured, \* with mixed GM/WM lesions covering larger areas and having a more prominent microglial reaction than \*\* pure GM lesions (94). Others have been documented similar findings with the proportion of MS cases exhibiting hippocampal demyelination ranging between 55% and 62% with around 30% of the total hippocampal area afflicted (76, 168). No hippocampal subregion appears to be spared (124, 168). Subpial and subependymal demyelination is commonly seen. A

contribution of hippocampal demyelination to cognitive dysfunction is not certain although mixed hippocampal lesions have been shown to be overrepresented in MS cases with cognitive impairment compared with those without (94). Whether this finding points toward a site-specific phenotypic consequence of demyelination or merely reflects a more severe global burden of disease is not known (Figure 2).

**Hippocampal inflammation** Hippocampal inflammation is not prominent. In one series, the majority of demyelinated lesions were chronic inactive (25/35, 71.4%), with only 9/35 (25.7%) lesions demonstrating microglial activation at their borders. Only one acute lesion was identified, which demonstrated microglial activation and perivascular CD3 T-cell infiltration. Microglial activation and T-cell inflammation in non-lesional areas is not significantly increased in MS compared with controls (168). CD3+ T-cell and HLA class II+ cell infiltration is noted in the choroid plexus and meninges but its relationship to underlying subependymal and subpial lesions, respectively, is not certain. These findings suggest that chronic inflammation of the hippocampal parenchyma or overlying meninges/choroid plexus appears an unlikely candidate for progressive cognitive impairment in MS.

**Hippocampal neuronal and synaptic loss** As noted above, GM structures appear susceptible to neuronal loss. However, the results on this aspect of hippocampal pathology have been conflicting. Papadopoulos *et al* reported that neuronal density was significantly decreased in CA1 (by 27%) and CA2-3 (by 29.7%) in MS cases compared with controls with no differences between the groups noted in CA4 (168). Neuronal atrophy in MS cases was restricted to the CA1 region, a finding not influenced by the extent of HLA class II+ cell expression. Correlations between global hippocampal cross-sectional area and neuronal density and size independent of lesions have fueled the notion that hippocampal atrophy is secondary to neuronal loss and atrophy (168). These observations contrast with those of Dutta and colleagues who found variable, but nonsignificant, reductions (10%–20%) in neuronal densities in demyelinated CA1, CA3 and CA4 hippocampal subfields in MS cases compared with controls (76).

Findings on synaptic loss in the MS hippocampus have yielded more consistent results. In their investigation, Papadopoulos revealed a 46.1% reduction in synaptophysin immunolabeled neuropil in CA4 in MS cases compared with controls, without correlation to the extent of demyelination, HLA class II+ inflammation or neuronal loss (168). Dutta *et al* compared synaptophysin-positive punctae in myelinated vs. demyelinated CA1 and dentate gyrus subregions. Loss of myelin was associated with significant reductions in synaptic density in both hippocampal areas (CA1—1.85-fold reduction; dentate gyrus—2.4-fold reduction). An elegant extension to this work revealed decreased expression of neuronal mRNA and proteins associated with axonal transport, glutamate neurotransmission and homeostasis, and memory and learning in demyelinated hippocampi (76, 77). Further experiments unraveled that demyelination altered neuronal gene expression and function by modulating levels of gene regulatory microRNAs in the hippocampus. Memory dysfunction in an animal model of hippocampal demyelination with similar consequent gene regulatory changes highlighted the functional significance of these findings though the mechanism by



which this occurs is not fully understood (77). The influence of altered cholinergic metabolism, known to be dysfunctional in the MS hippocampus and tied to memory dysfunction, on these neuronal gene expression changes, is worthy of exploration (124).

In short, hippocampal pathology is common in MS. The available clinicopathologic and animal model data implicate MS-related hippocampal damage as a factor leading to memory deficits. Although relationships between hippocampal pathology and cognitive dysfunction are already starting to emerge, much work is needed in understanding better the mechanism by which this structure is damaged and how this damage leads to cognitive troubles in MS. To this end, future studies should aim to implement more standardized methods to assess the nature, distribution and extent of hippocampal pathology in MS to not only facilitate useful comparisons between studies but also to allow meaningful correlations with cognitive measures obtained during life.

### Deep gray matter and cognitive impairment in MS

Radiographic studies have provided unequivocal evidence that atrophy in deep GM structures is prominent and contributes to cognitive dysfunction in MS. In the next section of this review, we will shift attention to the pathology of deep GM structures and evaluate their contribution to MS-related cognitive difficulties.

#### Thalamus

The thalamus is a highly integrated structure with connections to key cerebral cortical and subcortical structures critical for cognitive function, including hippocampus, amygdala, cingulate cortex, orbitofrontal cortex, retrosplenial cortex and inferior parietal lobule (18, 55, 150). It acts as an important gateway and has a multitude of reciprocal interactions between neocortical pyramidal, thalamic reticular nucleus and thalamocortical relay neurons central to normal oscillatory activity. Relevant to cognition, these diverse thalamic connections play a central role in arousal, executive function, emotional and episodic memory, spatial learning and memory, and recollective-based and familiarity-based recognition circuits (55). Therefore, damage to thalamic structures or their connections can potentially have a notable impact on cognitive function. As outlined above, radiographic studies have demonstrated that thalamic involvement is a feature of both early and late MS (108, 111) and contributes to cognitive disability in multiple domains. The neuropathologic features of MS-related thalamic injury and the implications on MS cognition will be presented here.

**Thalamic demyelination** Several pathologic studies confirm that demyelination in the thalamus is frequent with lesions commonly juxtaposed to the ependymal/CSF boundary (96, 106, 209, 211). In a study of 14 autopsy MS brains inclusive of RRMS and SPMS phenotypes, Vercellino *et al* observed lesions predominantly in periventricular areas of the thalamus involving cognitively relevant anterior and medial nuclei. Thalamic lesions affect both GM and WM structures to a similar degree, with mixed GM/WM lesions being relatively common (209). Where pure GM lesions arise, many respect the GM/WM boundary with lesional edges standing at the foot of where WM tracts and GM nuclei intersect (96, 209). While GM involvement in the thalamus is

proportionally less than that observed in the spinal cord, cingulate and cerebellum (96), it can be extensive in a subset of cases (211). The predisposition GM thalamic lesions to extend from subependymal surface suggest that soluble CSF factors may play a role in lesion formation here.

**Thalamic inflammation** Inflammation in the thalamus is characterized by a diffuse microglial infiltrate that is more pronounced in NAWM compared with NAGM structures (106, 209). In non-lesional matter, T- and B-cell infiltrates are rare and BBB disruption evidenced by fibrinogen deposition is absent. In most series, thalamic GM lesions are typically chronic with the extent of T-cell and macrophage/microglial infiltration relating to the stage of lesional activity (106, 209). Similar to other CNS regions, thalamic lesional WM is significantly more inflammatory than lesional GM, the former demonstrating more myelin-laden macrophages than the latter where these are uncommon. Evidence of fibrinogen deposition in WM greater than GM lesional areas is observed suggesting differing grades of BBB disruption that may explain the noted differences in inflammation, especially in the perivascular region (209). In a pooled analysis of deep GM pathology inclusive of thalamus, the extent of inflammation within deep GM lesions is intermediate compared with WM and cerebral cortex (106). While lymphoid-like structures have been demonstrated in the ventricular system of EAE (143), similar findings have not been demonstrated in human studies (144).

**Thalamic neuronal loss** Neuronal loss and axonal injury are elements of MS thalamic pathology. Cifelli and colleagues demonstrated significant reductions in total (21%) and medial dorsal nuclear thalamic (21%) volumes attributed to a similar reduction in neuronal density (22%) in GM areas distant from focal lesions (57). Reduction in neuronal area has also been described, also likely contributing to the observed thalamic volume changes (209). The extent of neuronal atrophy and loss is exaggerated in lesional compared with non-lesional matter and coincides with substantial glial cell loss found in a similar distribution. In contrast, no differences in synaptic density between lesional and non-lesional matter were observed (209). These findings implicate direct injury from inflammatory demyelination supported by the presence of axonal spheroids in both GM and WM parts of lesions (85, 209). That being said, the pathogenesis of neuronal/axonal injury in the thalamic circuitry is likely to be more complex with evidence of size-selective degenerative changes noted in a study exploring the anterior optic pathway and lateral geniculate nucleus (LGN). The selective neuronal size reduction in parvocellular, but not magnocellular, layers of the LGN, highlights how distinct areas of the thalamus may be differentially vulnerable to degeneration depending on their unique connectivity and cell composition (84). The relative contribution of retrograde degeneration, transynaptic reactive change, cytotoxic injury secondary to inflammatory cells or diffusible mediators to thalamic neuronal pathology remains unknown, although recent post-mortem MRI studies have shed light on this issue (122).

Research into thalamus pathologic correlates of cognitive decline has thus far been limited. The complex anatomic architecture of the thalamus, lack of systematic study of cognitively relevant thalamic nuclei and unavailable clinical neuropsychology metrics further complicate the established biases of post-mortem studies. Despite



these limitations, the demyelinating, inflammatory and neurodegenerative profiles of the anterior and medial dorsal nuclei of the thalamus is well established. Other thalamic nuclei are likely implicated but human neuropathological evidence is lacking. Given that these thalamic nuclei relay to limbic and prefrontal cortical structures, their contribution to impaired processing speed, memory impairment and executive dysfunction is likely, a notion supported by the previously presented radiographic studies.

### Basal ganglia

The basal ganglia consist of the striatum (composed of the caudate and putamen) and the globus pallidus (146). These deep GM nuclei receive inputs from intralaminar nuclei of the thalamus and several cortical regions, including frontal, inferotemporal and posterior parietal cortex to participate in parallel and partially segregated motor, oculomotor, cognitive and limbic circuits (19). There are several cortico-basal ganglionic “loops,” subsets of which connect the basal ganglia with dorsolateral prefrontal cortex, lateral orbitofrontal cortex and anterior cingulate/medial orbitofrontal cortices (148). These frontal cortical regions are involved in executive function (eg, planning and attention), rule-based learning and working memory, cognitive domains affected in MS. As noted previously, the relevance of basal ganglia on MS cognitive dysfunction is evidenced by strong correlations between MRI T<sub>2</sub> hypointensities in caudate, globus pallidus and putamen and measures of cognitive dysfunction (38). Despite the anatomic relevance of the basal ganglia to cognitive function, there have been few pathological studies examining MS-related pathology in this structure. We will present the available evidence here.

**Basal ganglionic demyelination** Basal ganglionic structures demonstrate demyelination, but there is considerable variation in the extent to which they are affected. In several reports, the caudate nucleus appears most susceptible to demyelination, especially in periventricular areas (106, 209). This is in contrast to the putamen and globus pallidus where demyelination is more restricted and typically centered on blood vessels (106, 209). In a combined analysis of several deep GM structures (inclusive of basal ganglia, thalamus and hypothalamus), the median percentage of deep GM demyelination was 3.1% (range 0%–26.8%) in acute MS, 14.5% (range 0.2%–31.6%) in RRMS, 6.0% (range 0%–46.4%) in secondary progressive MS and 9.9% (range 0%–91.3%) in primary progressive MS. Disease course and sex do not appear to influence the extent of demyelination in these deep GM structures (106). The majority of basal ganglionic lesions are mixed (ie, involving both GM and WM), with a greater proportion of the lesions in the caudate appearing “active” compared with putamen and globus pallidus in one series (209). Evidence of BBB disruption, inferred by fibrinogen deposition, is observed in both GM and WM components of deep GM lesions, being less pronounced in the former. The extent of deep GM demyelination appears to correlate with that found in the cerebral cortex in some studies (209) and appears to contribute to clinical deficits in an additive fashion when considered with cerebral cortical and WM lesion burden (106) (Figure 2).

**Basal ganglionic inflammation** Consistent with other reports on deep GM, including thalamus, basal ganglia demonstrate an

intermediate amount of inflammation in comparison to cerebral cortex and WM in both normal appearing and lesional tissue (106, 209). As typical of other brain regions, there is a gradient of T-cell inflammation (perivascular and parenchymal) being most severe in active deep GM lesions, followed by inactive deep GM lesions, and normal appearing GM. Microglial and macrophage inflammation is most enhanced in active deep GM regions where coincident iNOS expression and severe signs of oxidative injury in neurons, axons and oligodendrocytes may be seen. Iron deposition, as visualized by Turnbull blue staining method, is primarily found on oligodendrocytes and myelin fibers in normal appearing GM, which on demyelination, is mostly concentrated within microglia at lesional borders. Deep GM lesional inflammatory activity appears to decline significantly with advancing age (106).

**Basal ganglionic neuronal and synaptic loss** Neuronal loss and atrophy are features of basal ganglionic pathology in MS. In a comprehensive survey by Haider *et al*, abundant signs of neuronal/axonal injury as evidenced by neuronal cytoplasmic inclusive of oxidized phospholipids and  $\beta$ -APP positive axonal spheroids, were found in non-lesional areas of all deep GM nuclei. Neuronal loss was significantly reduced in normal appearing GM in MS (median 86.6 neurons/mm<sup>2</sup>; range 29.5–165.8 neurons/mm<sup>2</sup>) compared with matched controls (median 151.0 neurons/mm<sup>2</sup>; range 86.8–223.1 neurons/mm<sup>2</sup>;  $P < 0.001$ ) (106). While this group did not find any statistically significant differences in neuronal density between lesional and non-lesional deep GM, Vercellino *et al* found a 35.5% reduction in neuronal density in demyelinated vs. non-demyelinated caudate nucleus (209). This discrepancy between studies is not clear; however, differences in cohorts, sample size, counting methodology and precise deep GM regions studied may be contributory. No significant differences in synaptic density have been reported although further work in this area is required (209). Glial cells, implicated in neuronal trophic support, were also significantly reduced in the caudate by up to 70% (209). Neuronal density reductions in deep GM nuclei significantly associate with EDSS scores, with the impact on objective cognitive outcomes remaining unexplored (106).

Taken together, basal ganglionic pathology is a core feature of MS pathology. Given the intricate involvement of these deep GM nuclei in cortico-basal ganglionic loops with cortical regions implicated in memory and executive function, it is likely these structures contribute to cognitive decline in MS. Future pathological studies evaluating the contribution of demyelination, inflammation and neuronal/axonal pathology in the MS basal ganglia to cognitive decline would fill an important knowledge gap.

## DETERMINANTS OF COGNITIVE IMPAIRMENT: WHITE MATTER? GRAY MATTER? OR BOTH?

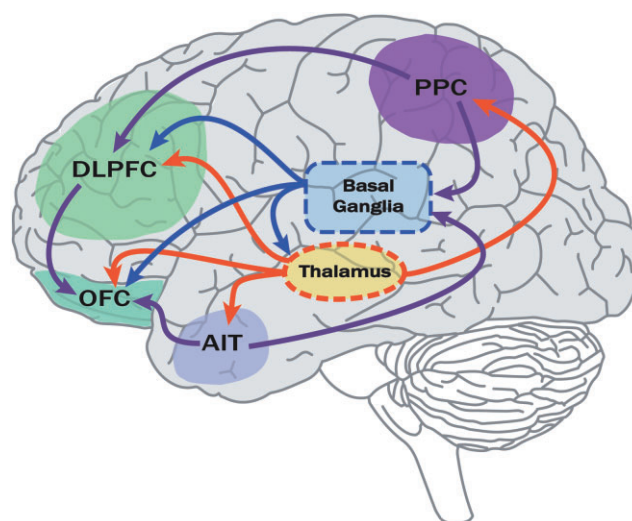
This review has thus far shown how MS pathology is multifaceted with cortical, deep GM and WM structures being significantly impacted. Given the intricate connectivity of these structures, it is likely that each contributes uniquely (and additively) to MS-related cognitive impairment. Further, it is feasible that pathology of one structure may affect the pathology in the other. This concept was borne out in a recent post-mortem imaging study

investigating two thalamo-cortical projection systems, namely lateral geniculate nucleus to primary visual cortex and mediodorsal nucleus to prefrontal cortex (122). The authors demonstrated that within the anatomically distinct thalamocortical projection systems, cerebral cortical thickness correlated significantly with myelination in the connected WM tract and neuronal density in the connected thalamic nucleus. Estimates of total lesion burden impinging on the connecting WM structures did not associate with the severity of pathology within each of the thalamo-cortical projection systems studied. These results highlight that nerve cell or axonal loss in a specific brain area can lead to anterograde or retrograde degeneration in connected brain areas. The consequences of this “tract-specific” pattern of MS neurodegenerative pathology are not clear. However, anterograde and retrograde neurodegeneration events are known to trigger microglial activation in areas distant to the initial insult (ie, focal inflammatory demyelination lesion). This can, in turn, propagate an inflammatory cascade leading to free radical generation and oxidative stress, factors known to compromise oligodendrocytes and the neuronal-axonal unit (105, 133). In short, one can deduce that damage to interconnected GM and WM structures can lead to a tract-specific neurodegenerative process with resultant inflammatory change that has the potential to further propagate neuronal-axonal demise. Should these tracts be involved in cognition, such as the dorsomedial thalamic and prefrontal system, then cognitive decline would be expected to ensue. Pathology targeting either GM and/or WM structures appear important to this outcome (Figure 3).

How do these findings translate into a possible mechanism of cognitive impairment in MS? In early disease stages, where focal inflammatory demyelination is thought to predominate, GM and/or WM lesions may disrupt interconnected cognitive pathways (Figure 3). Given the bias toward WM pathology in early disease stages (130), this could lead to subtle changes in subcortical cognitive function often encountered early on, with psychomotor slowing and inattention being common manifestations. In later disease stages, diffuse cortical and WM pathology may overwhelm the modest contribution of focal inflammatory demyelinating lesions leading to cognitive deficits typical of degenerative dementias, such as aphasia, apraxia and agnosia (130, 176). This model parallels that, which is seen in mixed dementia syndromes where, for example, the cognitive manifestations secondary to subcortical small vessel disease are later dominated by AD pathology (81). Where cognitive dysfunction is a presenting or early clinical symptom of MS, early extensive cerebral cortical inflammation and demyelination and/or disruption of interconnected cognitive pathways remain leading suspects (142).

## CONCLUSIONS

MS cognitive impairment is common, can occur at any disease stage and has a major impact on quality of life. Accordingly, cognitive batteries have become a staple of the clinical armamentarium used to evaluate patients with MS. Clinical neuropsychological studies have painted a clear picture of the range of cognitive deficits encountered in MS, and importantly, have laid the foundation to our understanding of the clinical consequences of disease gleaned from radiographic and pathologic studies. MRI studies have been monumental in dissecting the contribution of GM and WM pathology to MS cognitive impair-



**Figure 3.** Connectivity in the brain and cognitive function. Cognitive function relies on complex interconnections between cortical (cerebral cortex and hippocampus) and deep gray matter structures (eg, thalamus and basal ganglia), with adjoining white matter tracts being important conduits. In the cortex, pyramidal neurons connect cortical structures (cortico-cortical) or project to subcortical areas to form functionally distinct and temporally coordinated networks (purple lines). Various cortical regions are particularly relevant for cognition, including DLPFC (eg, working memory, planning, and cognitive flexibility), OFC (eg, decision making and adaptive learning), PPC (eg, spatial memory and attention) and AIT (eg, semantic memory) to name a few. The thalamus is a highly integrated structure with connections to several cerebral cortical and subcortical structures important for cognitive function (orange lines). The basal ganglia receive inputs from the intralaminar nuclei of the thalamus and several cortical regions, including frontal, inferotemporal and posterior parietal cortex and form several cortico-basal ganglionic loops important for cognitive function, including executive function, rule-based learning and working memory (blue lines). The hippocampal formation (not shown) forms a circuit with neocortical and subcortical structures and subserves diverse functions critical for cognition, including memory formation, maintenance and retrieval being key elements. Although shown with arrows, connections may be in both directions and produce feedforward cycles. AIT = anterior inferior temporal cortex; DLPFC = dorsolateral prefrontal cortex; OFC = orbitofrontal cortex; PPC = posterior parietal cortex.

ment but have been limited by inadequate resolution to detect disease in brain areas critical for cognition, such as cerebral cortex. This void has been filled, in part, by pathological studies, which have systematically evaluated the nature, extent and distribution of GM and WM pathology although the standard biases of post-mortem studies apply. The conflation of clinical, radiographic and pathologic data suggests that both GM and WM pathology play unique and likely additive roles in the pathogenesis of MS-related cognitive impairment. The relative contributions of GM and WM pathology, the interplay of inflammation and AD-like pathology, and the influence of genetic and environmental factors on MS cognitive decline are just a few of the many questions that warrant pursuit. Only through the study of prospective, longitudinally followed population-based cohorts with standardized clinical, paraclinical, neuropsychometric and radiographic outcomes

obtained during life and post-mortem tissue available after death will meaningful answers to these questions surface.

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## AUTHORS' CONTRIBUTIONS

The specific authors were involved in the following aspects of the review: review design (GD and SM), data gathering (GD, RY, HB, SM), wrote the manuscript (pathology sections: GD, RY, and HB; clinical and radiology sections: SM), decided to publish the paper (GD and SM).

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